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Sarah_Loftus@americanchemistry.com on 11/19/2002 11:34:20 AM

To: Rtk Chem/DC/USEPA/US@EPA, oppt.ncic@epamail.epa.gov
cc:

Subject: HERTG Succinimide Dispersants HPV submission

Test Plan Submission from the American Chemistry Council Petroleum Additives HERTG - HPV Registration Number

Three documents (1. cover letter, 2. test plan and 3. robust summaries) are attached to this e-mail for the HERTG HPV Succinimide Dispersants group. If you have any questions or comments, please feel free to contact me. Below, my contact information is listed. Thank you very much. Sarah McLallen

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(See attached file: Group 25.zip)

Group 25.zip

November 19, 2002

By Mail
Christine Todd Whitman, Administrator
US EPA
PO Box 1473
Merrifield, VA 22116

Attn: Chemical Right-to-Know Program – Test Plan Submission from HERTG
Registration Number

Dear Administrator Whitman:

The American Chemistry Council Petroleum Additives Panel (Panel) Health, Environmental, and Regulatory Task Group (HERTG) submits for review and public comment its test plan report, as well as related robust summaries, for the *"Succinimide Dispersants"* under the Environmental Protection Agency's High Production Volume (HPV) Chemical Challenge Program. The HERTG understands that there will be a 120-day review period for the test plan report and that all comments generated by or provided to EPA will be forwarded to the HERTG for consideration.

The succinimide dispersants, which are used as petroleum lubricant additives, are characterized by having structural similarities and limited reactivity, low biological activity, and limited water solubility. Based upon the data reviewed in the attached report, the HERTG concludes that the physicochemical and toxicological properties of the proposed succinimide dispersants group are similar and follow a regular pattern as a result of structural similarity. The two chemicals in the succinimide dispersant group are as follows:

- 2,5-Pyrrolidinedione,1-[2-[[2-[[2-[(2-aminoethyl)amino]ethyl]amino]ethyl]amino] ethyl]-, monopolyisobutenyl derivatives – (CAS # 67762-72-5), referred to as "mono alkenyl succinimide derivative".
- Amines, polyethylenepoly-, reaction products with succinic anhydride polyisobutenyl derivatives – (CAS # 84605-20-9), referred to as "bis alkenyl succinimide derivative".

The test plan for the succinimide dispersants indicates that no further testing or computer modeling is required.

Thank you in advance for your attention to this matter. If you have any questions regarding the test plan report or the robust summaries, or HERTG's activities associated with the Challenge Program, please contact Sarah McLallen at 703-741-5607 (telephone), 703-741-6091 (telefax) or Sarah_McLallen@americanchemistry.com (e-mail).

Sincerely yours,

Courtney M. Price
Vice President, CHEMSTAR

cc: HERTG members

HIGH PRODUCTION VOLUME (HPV)

CHALLENGE PROGRAM

TEST PLAN

For

SUCCINIMIDE DISPERSANTS

Prepared by
The American Chemistry Council
Petroleum Additives Panel
Health, Environmental, and Regulatory Task Group

October 9, 2002

**LIST OF MEMBER COMPANIES IN THE
HEALTH, ENVIRONMENTAL AND REGULATORY TASK GROUP**

The Health, Environmental, and Regulatory Task Group (HERTG) of the American Chemistry Council Petroleum Additives Panel includes the following member companies:

B.P. PLC

Chevron Oronite Company, LLC

Crompton Corporation

Ethyl Corporation

ExxonMobil Chemical Company

Ferro Corporation

Infineum

The Lubrizol Corporation

Rhein Chemie Corporation

Rhodia, Inc.

EXECUTIVE SUMMARY

The American Chemistry Council Petroleum Additives Panel Health, Environmental, and Regulatory Task Group (HERTG), and its member companies, hereby submit for review and public comment their test plan for the *"Succinimide dispersant"* category of chemicals under the United States Environmental Protection Agency High Production Volume (HPV) Chemical Challenge Program. This report should be read in its entirety in order to obtain an understanding of the chemical category and proposed testing.

Succinimide Dispersant Category. Relying on several factors specified in the EPA guidance document on "Development of Chemical Categories in the HPV Challenge Program," in which use of chemical categories is encouraged, the following two closely related chemicals constitute a chemical category:

- 2,5-Pyrrolidinedione,1-[2-[[2-[[2-[(2-aminoethyl)amino]ethyl]amino]ethyl]amino] ethyl]-, monopolyisobutenyl derivatives – (CAS # 67762-72-5), referred to as "mono alkenyl succinimide derivative".
- Amines, polyethylenepoly-, reaction products with succinic anhydride polyisobutenyl derivatives – (CAS # 84605-20-9), referred to as "bis alkenyl succinimide derivative".

Structural Similarity. A key factor supporting the classification of these chemicals as a category is their structural similarity. All substances in this category consist of a polyisobutylene succinic anhydride structure with polyethylene polyamine substituent groups.

Similarity of Physicochemical Properties. The similarity of the *physicochemical properties* of these substances parallels their structural similarity. All are dark colored viscous liquids intended for use as components in finished lubricating oils. The use of these substances in finished lubricants requires that they be stable under high temperatures (>100^{o}C). Their low volatility is due to their low vapor pressure, high viscosity, and relatively high molecular weights. The existing information for these substances indicates that they have low water solubility.

Fate and Transport Characteristics. Members of this category have been shown to be poorly biodegradable. Since the members of this category have low water solubility, hydrolysis testing is technically unfeasible. Furthermore, members of the category are resistant to hydrolysis because they lack hydrolyzable moieties. This makes hydrolysis modeling unnecessary. Photodegradation is not expected to cause significant physical degradation of succinimide dispersants. However, computer-modeled data will be developed to adequately characterize the potential atmospheric oxidation potential for members of this category. These substances are not expected to partition into water or into air if released into the environment due to their low water solubility and low vapor pressure, and computer-modeled environmental partitioning data indicates that these substances will partition into soil and sediment.

Toxicological Similarity. Review of existing published and unpublished test data for succinimide dispersants shows the *aquatic and mammalian toxicity* of the two substances within this category are similar and are of a low concern.

> *Aquatic Toxicology.* Data on acute fish toxicity, acute invertebrate toxicity, and alga toxicity were reviewed, and the findings indicate little to no toxicity when appropriate test methods are used. Therefore, the category has been adequately tested for acute aquatic toxicity, and no additional testing is necessary.

Mammalian Toxicology - Acute. Data on acute mammalian toxicity were reviewed, and the findings indicate a low concern for acute toxicity. Data are available for both members of the category indicating that the category has been well tested for acute mammalian effects. Therefore, no additional acute mammalian toxicity testing is necessary.

Mammalian Toxicology - Mutagenicity. Data from bacterial reverse mutation assays and *in vitro* and *in vivo* chromosome aberration studies were reviewed. Data are available for both members of the category, and the findings indicate a low concern for mutagenicity. Therefore, the category has been adequately tested for mutagenicity, and no additional mutagenicity testing is necessary.

Mammalian Toxicology - Subchronic Toxicity. Data from repeated-dose toxicity studies were reviewed. No signs of toxicity were observed following repeated oral or dermal exposure. Data are available for both members of the category. Therefore, the category has been adequately tested for repeated-dose toxicity, and no additional testing is necessary.

Mammalian Toxicology - Reproductive and Developmental Toxicity. Data from a reproductive/developmental toxicity screening study were reviewed. No signs of reproductive or developmental toxicity were observed following repeated oral exposure. These findings can be bridged to the other member of the category. Therefore, the category has been adequately tested for reproductive and developmental toxicity, and no additional testing is necessary.

Conclusion. Based upon the data reviewed for this test plan, the individual physicochemical, environmental fate, and toxicological properties of the proposed succinimide dispersant category members are similar and/or follow a regular, predictable pattern based on structural similarity and can be grouped together.

Test Plan. The test plan for the succinimide dispersant category indicates that no further testing or computer modeling is required. As this test plan was developed, careful consideration was given to the number of animals that would be required for tests included in the proposed plan and conditions to which the animals might be exposed. In consideration of the concerns of some non-governmental organizations about animal welfare, the use of animals in this proposed test plan has been minimized.

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1.0 INTRODUCTION

In March 1999, the American Chemistry Council (formerly the Chemical Manufacturers Association) Petroleum Additives Panel Health, Environmental, and Regulatory Task Group (HERTG), and its participating member companies committed to address certain chemicals listed under the Environmental Protection Agency (EPA) High Production Volume (HPV) Chemical Challenge Program. This test plan follows up on that commitment.

Specifically, this test plan sets forth how the HERTG intends to address physico-chemical, environmental, aquatic and health effects testing information for the following substances:

- 2,5-Pyrrolidinedione,1-[2-[[2-[[2-[(2-aminoethyl)amino]ethyl]amino]ethyl]amino] ethyl]-, monopolyisobutenyl derivatives – (CAS # 67762-72-5), referred to as "mono alkenyl succinimide derivative".
- Amines, polyethylenepoly-, reaction products with succinic anhydride polyisobutenyl derivatives – (CAS # 84605-20-9), referred to as "bis alkenyl succinimide derivative".

An analysis of the available data on these chemicals supports the designation of the succinimide dispersants as a "chemical category" as provided in the EPA guidance document entitled, "Development of Chemical Categories in the HPV Challenge Program". This document provides the basis for that determination, indicates the findings of the data review process, and sets forth a proposed testing plan to satisfy parts of the required test battery for endpoints without data that would be considered adequate under the program.

EPA guidance on the HPV Chemical Challenge Program indicates that the primary purpose of the program is to encourage "the chemical industry . . . to voluntarily compile a Screening Information Data Set (SIDS) on all chemicals on the US HPV list." (EPA, "Development of Chemical Categories in the HPV Challenge Program," p. 1) At the same time, EPA recognizes that the "large number of chemicals to be tested [about 2800 HPV chemicals] makes it important to reduce the number of tests to be conducted, *where this is scientifically justifiable.*" (*Id.*, p. 1) [emphasis added] The next part of the guidance explains where this would be scientifically justifiable:

> One approach is to test closely related chemicals as a group, or category, rather than test them as individual chemicals. In the category approach, *not every chemical needs to be tested for every SIDS endpoint.* However, *the test data finally compiled* for the category must prove adequate to support a screening level hazard-assessment of the category and its members. That is, the *final data set* must allow one to estimate the hazard for the untested endpoints, *ideally* by interpolation between and among the category members. In certain cases, where toxicity is low and no upward trend is expected, extrapolation to the higher category members may be acceptable. (*Id.*, p. 1) [emphasis added].

EPA guidance goes on to state, "The use of categories is encouraged in the Challenge Program and will have a number of benefits." (*Id.,* p. 1) Among the benefits identified in the guidance for the use of categories are "a reduction in testing will result in fewer animals used to test a category of chemicals as opposed to doing each test on each individual chemical," and "there will be . . . economic savings since less testing may be needed for chemicals considered as a category." (*Id.*, p. 1) That guidance also states that categories "accomplish the goal of the Challenge Program – to obtain screening level hazard information – through the strategic application of testing to the category." (*Id*, p. 2)

A similarly stated intent "to reduce the number of tests to be conducted, *where this is scientifically justifiable*" was articulated by the Agency in its draft guidance document titled, "The Use of Structure Activity Relationships (SAR) in the High Production Volume Chemicals Challenge Program." [emphasis added].

The EPA "Chemical Categories" guidance sets forth a definition of what constitutes a "chemical category, for the purposes of the Challenge Program". Specifically, that definition states that a chemical category under the HPV Challenge Program "is a group of chemicals whose physicochemical and toxicological properties *are likely to* be similar *or* follow a regular pattern as a result of structural similarity." (*Op. Cit*., p. 2) [emphasis added].

According to the guidance, what is important is that the "structural similarities [among members of the group] *may* create a predictable pattern *in any* or all of the following parameters: physicochemical properties, environmental fate and effects, and human health effects." (Id., p. 2) [emphasis added]. Thus, it is not necessary for the chemicals in a category to be similar in all respects. Nor must there be conclusive proof that the chemicals in the postulated category will behave identically across all relevant parameters. All that is required for an acceptable category under the HPV Challenge Program is that there be a *likelihood* of similarity of physicochemical and toxicological properties or a *likelihood* that the chemicals will in some pertinent respect follow a regular pattern as a result of their structural similarity.

In identifying the succinimide dispersant category, the six-step process set out in the EPA guidance on category development was followed. As the information below indicates, the succinimide dispersant chemicals clearly satisfy the standards established in that guidance for use of a chemical category:

Step 1: group structurally similar chemicals into a putative category

Step 2: gather relevant published and unpublished literature for each member of the category

Step 3: evaluate the compiled data for adequacy in accordance with the EPA guidance documentation

Step 4: construct matrices of SIDS endpoints versus category members arranged so as to indicate the structural progression of the category (in this case, by increasing molecular weight)

Step 5: evaluate the data to determine whether there is a correlation between category members for each SIDS endpoint

Step 6: make available to EPA, and to the public for review, this test plan including the foregoing category definition and rationale and the following data assessment with the proposed testing scheme for the succinimide dispersants.

2.0 CHEMISTRY OF SUCCINIMIDE DISPERSANTS

2.1 DESCRIPTION

Succinimide dispersants consist of a polyisobutylene hydrocarbon chain connected to a polyethylene polyamine substituent group by a succinic anhydride linking group. The polyisobutylene portion of the molecule is a saturated branched hydrocarbon of that may vary from 950 to 2500 daltons in molecular

weight. The polyethylene polyamine substituent group may vary from diethylenetriamine to a "heavy polyamine", which contains from 5 to 10 ethylene amine groups. The chemical names and CAS numbers for the members of the succinimide dispersant category are presented in Table 1 and the chemical structures in Table 2.

These substances are prepared by reacting polyisobutylene succinic anhydride with a polyethylene polyamine in highly refined lubricating base oil. Thus the "active ingredients" are never isolated during the life cycle of these substances. This is done for two reasons: 1) the kinetics of the chemical reactions used in the manufacturing process are optimized when highly refined lubricating base oils are used as the reaction solvent, and 2) lubricant additives diluted in highly refined lubricating base oils are required to control viscosities during blending with other additives or with additional highly refined lubricating base oil to make finished lubricants. To meet the required viscosities for these substances, the concentration of highly refined lubricating base oil ranges from 25 wt% to 35 wt%.

There are two structural variables that influence the molecular weight of the category members and consequently their bioavailability and toxicity: the polyethylene polyamine and the isobutylene subtituents. As mentioned above, the polyethylene polyamine substituent group may vary from diethylenetriamine (102 daltons in molecular weight) to a "heavy polyamine" (231 to 446 daltons in molecular weight). Although the distribution frequency of the number of ethylene amine groups in heavy polyamine ranges from 5 to 10, the relative proportions of each distribution frequency are expected to be similar to meet industry performance requirements. However, the structural variable that has the greatest impact on the molecular weight of these molecules is the molecular weight of the polyisobutylene group. The carbon chain length of the 950 dalton polyisobutylene is approximately C68, and the carbon chain length of the 2200 dalton polyisobutylene is approximately C160. Linking more than one polyisobutylene succinic anhydride group to the polyethylene polyamine to form a "bis" molecule essentially doubles the molecular weight. As the alkyl carbon chain increases and molecular weight increases, bioavailability is expected to decrease. In addition, as the alkyl carbon chain length increases, water solubility is expected to decrease. Thus, aquatic toxicity is expected to decrease with increasing alkyl carbon chain length. Consequently, the members of this category are arrayed by increasing molecular weight, which is primarily dependent on polyisobutylene carbon chain length, and, to a lesser extent, on the number of ethylene amine groups in the polyethylene polyamine.

2.2 PHYSICOCHEMICAL PROPERTIES

The physicochemical properties of the members of the succinimide dispersant category are presented in Table 3. Succinimide dispersants, as manufactured and distributed in commerce in highly-refined lubricating base oil, are dark brown viscous liquids. These substances exist in the absence of base oil as idealized structures only. Attempts to "de-oil" succinimide dispersants result in substances that are solid materials that do not retain their original chemical structure and physico-chemical properties that are critical for performance. Therefore, the measured physico-chemical properties presented in Table 3 are derived from a mixture of the succinimide dispersant in highly refined lubricating base oils, and the modeled physico-chemical data are based on the idealized structure.

2.2.1 Molecular Weight

The members of the category range in molecular weight from 1134 to 3160 daltons. The two structural variables that influence the molecular weight of the category members have been

discussed above. Due to the influence of molecular weight on water solubility and bioavailability, the members of the category are arrayed in order of increasing molecular weight in Tables 3-9.

2.2.2 Specific Gravity

Available specific gravity data are presented in Table 3. The specific gravity of the members of the category as manufactured in highly refined lubricating base oil is approximately 0.91 @ 60^{o}F.

2.2.3 Melting Point and Boiling Point

Succinimide dispersants, as manufactured in highly refined lubricating base oils, are liquid at ambient temperature. The use of these substances in finished lubricants requires that they be thermally and chemically stable under high temperatures (>100^{o}C). Typically, the petroleum base stocks in these substances boil at temperatures above 300^{o}C. Modeling data for the theoretical "de-oiled" substances indicates that the boiling point is 841.5 ^{o}C to 1271.5 ^{o}C.

2.2.4 Vapor Pressure and Viscosity

As mentioned above, attempts to "de-oil" succinimide dispersants result in substances that are solid materials. Thus, the vapor pressure of the succinimide dispersants as manufactured in highly refined lubricating base oil can be estimated from the vapor pressure of the base oil in which they are manufactured. Typically, highly refined lubricating base oils have a low vapor pressure, < 10^{-10} Pa @ 25^{o}C (Table 3). In addition, the viscosity of these substances is also dependent on that of the highly refined lubricating base oil used in their manufacture. Measured viscosities range from 1100 to 1330 cSt @ 40^{o}C (Table 3). Thus, the low volatility of the members of the succinimide dispersants category is due to their low vapor pressure, high viscosity, and high relative molecular weights.

2.2.5 Water Solubility and Octanol-Water Partition Coefficients

The water solubility of a representative succinimide dispersant, mono alkenyl succinimide derivative (CAS # 67762-72-5), was measured at 0.125 mg/L. This value indicates that succinimide dispersant are generally regarded to be insoluble in water. A log P value of 6.7 was also determined for this derivative (Table 3).

3.0 USES OF SUCCINIMIDE DISPERSANTS

Succinimide dispersants are used to formulate finished lubricating oils including all types of automotive and diesel engine crankcase oils, air and water-cooled two-cycle engine oils, natural gas engine oils, marine trunk piston engine oils, and medium-speed railroad diesel engine oils. They are used as ashless dispersants to inhibit colloidal particle-to-particle aggregation by an adsorbed film mechanism, and they solubilize oil-insoluble liquids. Succinimide dispersants are generally sold to finished oil blenders in additive packages, where the concentration ranges from 5 to 50 wt.%. These additive packages are then blended into finished oils where the typical concentration of succinimide dispersant ranges from 0.5 to 10 wt.% in the finished oil.

Succinimide dispersants in this category are manufactured at plants owned by members of the HERTG and blended into additive packages at plants owned by members of the HERTG and their customers. Finished lubricants are blended at facilities owned by HERTG's customers. Additive packages are shipped to customers in bulk in ships, isocontainers, railroad tank cars, tank trucks or in 55-gallon steel drums. The bulk additive packages are stored in bulk storage tanks at the customer blending sites. Finished oils are blended by pumping the lubricating oil blend stocks and the additive package from their storage tanks through computer controlled valves that meter the precise delivery of the components into a blending tank. After blending, the finished lubricant products are sold in bulk and shipped in tank trucks to large industrial users, such as manufacturing facilities and facilities that service truck fleets and passenger motor vehicles. Finished lubricants are also packaged into 55-gallon drums, 5-gallon pails, and one-gallon and one-quart containers for sale to smaller industrial users. Sales of lubricants in one-gallon and one-quart containers to consumers at service stations or retail specialty stores also occur.

Based on these uses, the potentially exposed populations include (1) workers involved in the manufacture of succinimide dispersants, blending them into additive packages, and blending the additive packages into finished lubricants; (2) quality assurance workers who sample and analyze these products to ensure that they meet specifications; (3) workers involved in the transfer and transport of succinimide dispersants, additive packages or finished lubricants that contain them; (4) mechanics who may come into contact with both fresh and used lubricants while working on engines or equipment; (5) gasoline station attendants and consumers who may periodically add lubricating oil to automotive crankcases; and (6) consumers who may change their own automotive engine oil. The most likely route of exposure for these substances is skin and eye contact. Manufacturing, quality assurance, and transportation workers will likely have access to engineering controls and wear protective clothing to eliminate exposure. Mechanics wear protective clothing, but often work without gloves or eye protection. Gasoline station attendants and consumers often work without gloves or other protective equipment. The most likely source of environmental exposure is accidental spills at manufacturing sites and during transport.

4.0 EVALUATION OF AVAILABLE PUBLIC AND COMPANY DATA

4.1 Environmental Fate Data

4.1.1 Physicochemical Properties Relevant to Environmental Fate

In order to understand the environmental fate of a substance, one must understand how that substance can potentially partition among environmental compartments (i.e., air, soil, sediment, suspended sediment, water, and biota). The physicochemical properties of a substance influence the way in which a substance will degrade. The important environmental degradation pathways include biodegradation, hydrolysis, and photodegradation. Biodegradation is a measure of the potential of a compound to be degraded by microorganisms. Hydrolysis is a reaction in which a water molecule or hydroxide ion substitutes for another atom or group of atoms present in an organic molecule. Photodegradation is the degradation of a chemical compound as a result of absorption of solar radiation.

The physicochemical properties of the parent substance will influence the way in which these substances may partition among environmental compartments. Substances characterized by a low vapor pressure do not partition into air to any great extent. Similarly, substances that are characterized by low water solubility do not partition extensively into water. Substances that do not partition into air and water to any great extent tend to partition into soil and sediments.

4.1.2 Biodegradability

4.1.2.1 Test Methodologies

Chemical biodegradation involves a series of microbially-mediated reactions that may require many kinds of microorganisms acting together to degrade the parent substance. There are several standard test methods, which measure primary degradation (i.e., loss of parent chemical) or ultimate degradation (i.e., complete utilization of the substance to produce carbon dioxide, water, mineral salts, and microbial biomass). Primary degradation can be determined analytically by measuring dissolved organic carbon (DOC) for water-soluble chemicals, infrared absorbance, or by a chemical-specific detection method. Ultimate degradation (also called mineralization) can be determined by measuring oxygen consumption or carbon dioxide evolution relative to the theoretical levels that can be achieved based on an elemental analysis of the chemical under investigation.

4.1.2.2 Summary of Available Data

Biodegradation data for the succinimide dispersant category are summarized in Table 4. One member of the category have been adequately tested.

The Modified Sturm Test (OECD Guideline 301B, *CO_2 Evolution Test*) was used to evaluate the biodegradability of bis alkenyl succinimide derivative (CAS # 84605-20-9). After the 28-day test, the extent of biodegradation was 16% based on carbon dioxide evolution.

4.1.2.3 Data Assessment and Test Plan for Biodegradability

One biodegradation test has been conducted on one of the two members of the succinimide dispersant category, and the results indicate that this substance is poorly biodegraded.

Adequate biodegradation data exist for one of the two substances in the succinimide dispersant category. These data will be bridged to both category members due to the presence of predominantly branched polyisobutylene chains in these substances, thereby characterizing the biodegradability of the entire category.

4.1.3 Hydrolysis

4.1.3.1 Test Methodologies

The potential for a substance to hydrolyze in water is assessed as a function of pH (OECD Guideline 111, *Hydrolysis as a Function of pH*[1]). When an organic molecule undergoes hydrolysis, a nucleophile (water or hydroxide ion) attacks an electrophile and

[1] Organization for Economic Cooperation and Development (OECD) (1993) OECD Guidelines for Testing of Chemicals. OECD. Paris, France.

displaces a leaving group (e.g., halogen, phenoxide).[2] Potentially hydrolyzable groups include alkyl halides, amides, carbamates, carboxylic acid esters and lactones, epoxides, phosphate esters, and sulfonic acid esters[3]. The lack of a suitable leaving group renders compounds resistant to hydrolysis.

4.1.3.2 Summary of Available Data

There are no published or unpublished hydrolysis studies for members of the succinimide dispersant.

4.1.3.3 Data Assessment and Test Plan for Hydrolysis

Although these substances contain amide functional groups that are susceptible to hydrolytic degradative mechanisms[3], testing these substances for hydrolysis as a function of pH is not needed to adequately evaluate this endpoint. A technical discussion will be developed as a robust summary that characterizes the potential for succinimide dispersants in this category to undergo hydrolysis. Therefore, no hydrolysis testing is proposed for the HPV Challenge Program (Table 4).

4.1.4 Photodegradation

4.1.4.1 Test Methodologies

Photodegradation can occur as a result of direct and indirect mechanisms. A prerequisite for direct photodegradation is the ability of one or more bonds within a chemical to absorb ultraviolet (UV)/visible light in the 290 to 750 nm range. Light wavelengths longer than 750 nm do not contain sufficient energy to break chemical bonds, and wavelengths below 290 nm are shielded from the earth by the stratospheric ozone layer. In comparison, indirect photodegradation also requires light energy as well as a series of chemical reactions that include a reaction of the parent molecule with hydroxyl radicals.

Direct photochemical degradation occurs through the absorbance of solar radiation by a chemical substance. If the absorbed energy is high enough, then the resultant excited state of the chemical may lead to its transformation. Simple chemical structures can be examined to determine whether a chemical has the potential for direct photolysis in water. First order reaction rates can be calculated for some chemicals that have a potential for direct photolysis using the procedures of Zepp and Cline (1977). [Zepp, R. G., and D. M. Cline. 1977. Rates of Direct Photolysis in the Aqueous Environment. Environ. Sci. Technol. 11:359.366.]

To develop information that characterizes the potential of the members in this category to undergo direct photochemical degradation, the existing subtance chemical composition has been evaluated and a subset of chemicals has been selected that adequately represent substances in this category.

Although substances in this category have a low potential to volatilize to air where they can react with hydroxyl radicals (OH-), photodegradation can be estimated using models

[2] W. Lyman et al. (1990) *Handbook of Chemical Estimation Methods*. Chapter 8.
[3] W.J. Lyman, W.F. Reehl, and D.H. Rosenblatt. (1982) Handbook of Chemical Property Estimation Methods. McGraw-Hill Book Co. New York, NY, USA.

accepted by the US EPA. An estimation method accepted by the US EPA includes the calculation of atmospheric oxidation potential (AOP) to characterize this fate property.

The computer program AOPWIN (atmospheric oxidation program for Microsoft Windows) (EPIWIN, 1999) is used by the US EPA OPPTS (Office of Pollution Prevention and Toxic Substances). This program calculates a chemical half-life based on an overall OH- reaction rate constant, a 12-hr day, and a given OH- concentration.

4.1.4.2 Summary of Available Data

The atmospheric oxidation potential of the selected chemical structures for members of the succinimide dispersant category is presented in Table 2 of Appendix I. The modeling data indicates that the members of this category have a very low potential to photodegrade.

4.1.4.3 Data Assessment and Test Plan for Photodegradation

Modeling data indicate that members of the Succinimide Dispersant category do not undergo photodegradation in the environment. The potential for category members to undergo direct photodegradation has been adequately evaluated, and no further testing is required.

4.1.5 Fugacity Modeling

4.1.5.1 Modeling Methodologies

Fugacity-based multimedia fate modeling compares the relative distribution of chemicals among environmental compartments. A widely used model for this approach is the EQC model[4].

There are multiple levels of the EQC model. In the document, "Determining the Adequacy of Existing Data", EPA states that it accepts Level I fugacity modeling to estimate transport/distribution values. The EQC Level I model utilizes input of basic chemical properties, including molecular weight, vapor pressure, and water solubility to calculate percent distribution within a standardized environment. EQC Level III model uses these parameters to evaluate chemical distribution based on emission rates into air, water, and soil, as well as degradation rates in air, water, soil, and sediment.

4.1.5.2 Summary of Available Data

Fugacity-based multimedia fate modeling data for members of the succinimide dispersant category are presented in Table 3 of Appendix I. All of the members of this category have low vapor pressure and low water solubility, and the modeling data indicate that they will partition into soil and sediment.

4.1.5.3 Test Plan for Fugacity

The relative distribution of substances within this category among environmental compartments has been evaluated using the Level I model. Data developed using a Level I model indicate that the members of the Succinimide Dispersant category partition into

[4]. Mackay, D., A. Di Guardo, S. Paterson, and C. E. Cowan. 1996. Evaluating the Environmental Fate of a Variety of Types of Chemicals Using the EQC Model. Environ.

soil and sediment. Based on this assessment, the fugacity of the members of the category has been adequately evaluated, and no further work is required.

4.2. ECOTOXICOLOGY DATA

4.2.1 Aquatic Ecotoxicity Testing

4.2.1.1 Test Methodologies

Acute aquatic ecotoxicity tests are usually conducted with three species that represent three trophic levels in the aquatic environment: fish, invertebrates, and algae. The fish acute toxicity test (OECD Guideline 203, *Fish, Acute Toxicity Test*) establishes the lethality of a substance to a fish during a 96-hour exposure period. The acute invertebrate test (OECD Guideline 202, *Daphnia sp., Acute Immobilization Test and Reproduction Test*) establishes the lethality of a substance to an invertebrate, typically a daphnid (*Daphnia magna*), during a 48-hour exposure period. The alga growth inhibition test (OECD Guideline 201, *Alga, Growth Inhibition Test*) establishes the potential of a substance to inhibit alga growth, typically using the freshwater unicellular green algae, *Pseudokirchneriella subcapitata* (formerly called *Selenastrum capricornutum*), during a 96-hour exposure period.

Three test methodologies are commonly used to conduct aquatic toxicity tests; i.e., flow-through, static, and static renewal tests.

In *flow-through tests*, organisms are continually exposed to fresh chemical concentrations in each treatment level in the incoming water and there is greater assurance than with other test methods that the exposure levels and water quality remains constant throughout the test. Although flow-through testing is the preferred method, it is only applicable for chemicals that have adequate water solubility for testing.

In *static tests*, organisms are exposed in the test medium that is not replaced for the duration of the study. There is less assurance that the test concentrations will remain constant because test material can be adsorbed onto test chambers, degraded, volatilized, or otherwise changed during the test. Nevertheless, due to limitations of other test systems for non-volatile materials, the static test has been widely used, especially for testing organisms such as algae and *Daphnia*.

The *static-renewal test* is similar to a static test because it is conducted in still water, but the test solutions and control water are renewed periodically, usually every 24 hours. Daily test solution renewal provides a greater likelihood that the exposure concentrations will remain stable throughout the test. This is the preferred method for conducting aquatic toxicity tests for compounds such as the succinimide dispersants on fish. Daily renewals cannot be done in the algae test, and usually not in *Daphnia* tests, because the process of separation and replenishment would cause a discontinuity in the alga growth rate and it can stress, coat, or entrap *Daphnia* in any surface film during renewals. OECD considers the use of static test for *Daphnia* and algae, and the use of static renewal test for fish to be appropriate for testing poorly soluble chemicals like the succinimide dispersants provided

that test solution preparation uses water accommodated fraction or water soluble fraction methods.[7]

4.2.1.2 Test Solution Preparation

Succinimide dispersants are poorly water-soluble substances, and it is not possible to prepare exposure solutions for aquatic toxicity testing by direct addition of measured quantities of test material to water. Two methods[6] are used to prepare solutions of poorly water-soluble materials for aquatic toxicity testing:

- *Water accommodated fraction* (WAF) – This is a method in which the test solution contains only that fraction of the test material (organic phase) which is retained in the aqueous phase after a period of stirring long enough to reach equilibrium, followed by a sufficient time (1-4 hours) for phase separation. The WAF (aqueous phase) will contain soluble components of the test material at levels that will be dependent on the test material loading (the amount of material added to the aqueous medium). The resulting WAF is used in the aquatic toxicity test. Ideally, a WAF consists of a water-soluble extract of test material, but it can also include a stable micro-emulsion or contain small amounts of suspended matter.

- *Water soluble fraction* (WSF) – This is a method in which a WAF is either filtered, centrifuged, or allowed to settle for a greater length of time (24 hours) than with the WAF method to remove suspended matter from the aqueous phase before being used in the aquatic toxicity test.

4.2.1.3 Reporting Toxicity Results

In both WAF and WSF tests, test material concentrations are expressed as loading rates (i.e., defined as the weight of test material added per unit volume of test medium during WAF or WSF preparation)[7]. For fish tests, endpoints can be expressed as median lethal loading rate (LL_{50}) when lethal effects occur to 50% of the test population or in cases where no lethal effects are observed at all loadings tested, LL_0. In both cases, results can be expressed in mg/L and in studies where no lethality is observed, the result is expressed as LL_0 = the highest loading rate tested. For invertebrate and alga tests, endpoints are expressed as median effective loading rate (EL_{50}) or EL_0 in mg/L as discussed above.

Loading rates allow poorly water-soluble complex substances such as the succinimide dispersants to be compared to more readily soluble substances and /or pure chemicals on an equal basis. To allow comparison, the toxicity value is expressed as the amount of test material added per unit volume of water when preparing the WAF or WSF.

If test material exposure levels are analytically measured in the test, the endpoints can also be expressed as median lethal concentration (LC_{50}) or median effective concentration

[5] Organization for Economic Cooperation and Development (OECD) (2000). Guidance Document on Aquatic Toxicity Testing of Difficult Substances and Mixtures. OECD Environmental Health and Safety Publications, Series on Testing and Assessment No.23, Paris, France.

[6] American Society for Testing and Materials (1998) D6081-98, Standard Practice for Aquatic Toxicity Testing of Lubricants: Sample Preparation and Results Interpretation.

[7] Organization for Economic Cooperation and Development (OECD) (1999) Draft Guidance document on Aquatic Toxicity Testing of Difficult Substances. OECD, France.

(EC_{50}) in mg/L. EC/LC_{50}s are often not reported because it is very difficult to accurately measure test material exposure levels that can be below 1.0 mg/L.

NOTE: In this test plan, these results are reported as loading rates (EL/LL), to reflect the current reporting practices for the WAF method used in the tests. In the robust summaries, these data are presented as concentrations (EC/LC) as originally reported even though the test methods employed WAF preparation of test solutions without measurement of test material concentration.

4.2.2 Aquatic Toxicity of the Succinimide Dispersants

In general, the toxicity of a substance to an organism is limited by mechanisms of uptake and movement to target organs. Characteristics such as smaller molecular size and a lesser degree of ionization increase the ability of a substance to passively cross biological membranes. However, the soluble fraction of a compound in water represents the chemical fraction responsible for toxicity to aquatic organisms. Therefore, aquatic toxicity can be limited by the water solubility of a substance.

Data and preliminary modeling information indicates that all members of the succinimide dispersant category have low water solubility. The low water solubility suggests that the acute aquatic toxicity of these substances should be low due to limited bioavailability to aquatic organisms. However, the length of the alkyl side chains on these substances will influence their relative water solubility, and, hence, their relative toxicity.

4.2.2.1 Summary of Available Data

Acute aquatic ecotoxicity data for the succinimide dispersant category are summarized in Table 5. Both members of the category have been tested for acute aquatic toxicity in fish, daphnia and algae. A low order of toxicity was observed in all tests.

4.2.2.1.1 Fish Acute Toxicity

Both substances in the category were evaluated for acute toxicity to fish. The maximum test material loading rate was 1000 mg/L, and no mortality was observed in the studies. Overall, the LL_{50} for both substances from both studies were greater than 1000 mg/L, indicating a relatively low order of toxicity to fish.

4.2.2.1.2 Invertebrate Acute Toxicity

Both substances in the category were evaluated for acute toxicity to daphnids. The maximum test material loading rate was 1000 mg/L. Overall, the EL_{50} for the two substances were greater than 1000 mg/L, indicating a relatively low order of toxicity to daphnids.

4.2.2.1.3 Alga Toxicity

Both substances in the category were evaluated for algal growth inhibition. The maximum test material loading rate was 1000 mg/L. Overall, the EL_{50} for these substances was greater than 100 mg/L indicating a relatively low order of toxicity to algae.

4.2.2.2 Data Assessment and Test Plan for Acute Aquatic Ecotoxicity

In total, six adequate acute aquatic ecotoxicity studies have been conducted for the succinimide dispersant category. These studies involved all three trophic levels of aquatic

organisms and evaluated the acute aquatic ecotoxicity of both members of the category. The data demonstrate a low order of acute aquatic ecotoxicity. The similarity in the low order of toxicity for these substances is consistent with their similar chemical structure and physicochemical properties and supports the scientific justification of these twelve substances as a category within the HPV Challenge Program.

Adequate data for all three trophic levels exist for both substances in the category. Therefore, no additional testing is necessary.

4.3 MAMMALIAN TOXICOLOGY DATA

4.3.1 Physicochemical Properties Relevant to Mammalian Toxicity

Physicochemical properties of chemicals are useful for predicting the routes by which exposure may occur, and in some cases, the mechanism and extent of toxicological responses. The physicochemical properties of the succinimide dispersants that are presented in Table 3 show these substances are relatively high molecular weight, liquid substances with moderately high octanol/water partition coefficients and low water solubilities. These characteristics indicate that the succinimide dispersants are lipophilic, and thus, capable of passive diffusion across biological membranes. It would be predicted that upon oral exposure these chemical substances would be absorbed by the gastrointestinal tract. However, the structural and physical properties such as comparatively high molecular weight, the presence of long-chain alkyl moieties and poor water solubility is expected to impede the rate and extent of absorption of succinimide dispersants from dermal surfaces. In addition to the general considerations discussed above, the succinimide dispersants are high boiling point, low vapor pressure, high viscosity liquid components. As a result, these substances have a low propensity to form vapors or aerosols, and thus, unintentional exposure via inhalation is uncommon.

4.3.2 Acute Mammalian Toxicity of Succinimide Dispersants

4.3.2.1 Acute Toxicity Test Methodology

Acute toxicity studies investigate the effect(s) of a single exposure to a relatively high dose of a substance. Potential routes of exposure for acute toxicity assays include oral, dermal, and inhalation. Oral toxicity assays are conducted by administering test material to fasted animals (typically rats or mice) in a single gavage dose. Acute dermal toxicity tests are conducted by administering test material to the shaved skin on the back of the test animal (typically rats or rabbits) and allowing the test material to stay in contact with the skin application site for a specific duration (usually 24 hours). Acute inhalation toxicity assays are conducted by exposing test animals (typically rats) in a controlled atmosphere to a fixed air concentration of the test substance for a specific duration (typically 4 hours). The test material is either generated as a vapor or intentionally aerosolized into respirable particles, then metered into the exposure air at the desired concentration. Preferably, inhalation toxicity studies are conducted using either nose-only or head-only exposure to minimize potential confounding effects resulting from whole-body exposure. Whole body exposure may lead to over-prediction of inhalation toxicity hazard by increasing the body-burden of the test material through skin absorption or ingestion of test material as a consequence of grooming both during and after the inhalation exposure period.

Historically, lethality is a primary end-point of concern in acute toxicity studies, and the traditional index of oral and dermal potency is the median lethal dose that causes mortality in 50 percent of the test animals (LD_{50}). In acute inhalation studies, the traditional measurement of potency is the median lethal concentration of the test material in air that causes mortality in 50 percent of the test animals (LC_{50}). In addition to lethality, acute toxicity studies also provide insights regarding potential systemic toxicity through careful observation and recording of clinical signs and symptoms of toxicity as well as through detailed examination of tissues and organ systems.

Typically, acute oral and dermal toxicity studies are conducted using a limit dose of 5000 and 2000 mg/kg body weight, respectively, and acute inhalation toxicity studies are conducted using a limit dose of 5 mg/L for 4 hours (according to OECD and EPA testing guidelines). Prior to 1990, some acute dermal toxicity studies may have used a limit dose of 5000 mg/kg. Recently, harmonized EPA testing guidelines (August 1998) have set the limit dose for both oral and dermal acute toxicity studies at 2000 mg/kg body weight, while the recommended limit concentration for acute inhalation studies has been set at 2 mg/L for 4 hours. The limit dose test method minimizes the number of animals tested by exposing a single group of animals to a large dose (the limit dose) of the test substance. A test substance that shows little or no effects at the limit dose is considered essentially nontoxic, and no further testing is needed. If compound-related mortality is observed at the limit dose, then further testing may be necessary.

4.3.2.2 Summary of Available Data

Acute toxicity data for the succinimide dispersant category is summarized in Table 6. Both members of the category have been tested by the oral and dermal route of administration and demonstrate a low order of acute toxicity.

4.3.2.2.1 Acute Oral Toxicity

Both substances in the succinimide dispersant category have been adequately tested for acute oral toxicity. The acute oral LD_{50} for these studies in rats is greater than 5000 mg/kg (limit tests). Clinical signs observed following treatment included diarrhea and dark staining of the anal area. There were no significant necropsy findings. Overall, the acute oral LD_{50} for these substances was greater than 5000 mg/kg indicative of a relatively low order of lethal toxicity.

4.3.2.2.2 Acute Dermal Toxicity

Both substances in the succinimide dispersant category have been adequately tested for acute dermal toxicity. The acute dermal LD_{50} for these studies in rabbits and rats were greater than 2000 mg/kg (limit tests). Dermal application of the test materials to the skin of rabbits for 24 hours typically produced slight to well-defined erythema, which persisted through the first 7 days of the study and was characterized microscopically as dermatitis, hyperkeratosis, and acanthosis. Clinical signs in rabbits included reduced weight gain. There were no remarkable findings in rats. There were no remarkable macroscopic observations at necropsy. Overall, the acute dermal LD_{50} for these substances were greater than 2000 mg/kg indicative of a relatively low order of lethal toxicity.

4.3.2.3 Data Assessment and Test Plan for Acute Mammalian Toxicity

In total, four adequate acute toxicity studies have been conducted for both members of the succinimide dispersant category. These studies involved two species of laboratory

animals (rats or rabbits); two routes of exposure (oral and dermal); and evaluated the toxicity of both members of the category. The data consistently demonstrate a low order of acute toxicity. The overall low order of acute toxicity for these substances in combination with their similar chemical structure and physicochemical properties supports the scientific justification of these twelve substances as a category within the HPV Challenge Program.

Based on the results of these studies, the acute toxicity of the category has been evaluated adequately with respect to all acute toxicity endpoints, and no additional acute toxicity testing is proposed for the HPV Challenge Program.

4.3.3 Mutagenicity of the Succinimide Dispersant Category

4.3.3.1 Mutagenicity Test Methodology

Genetic toxicology is concerned with the effects of substances on genetic material (i.e., DNA and chromosomes). Within genetic material, the gene is the simplest functional unit composed of DNA. Mutations are generally non-lethal, heritable changes to genes that may arise spontaneously or because of xenobiotic exposure. Genetic mutations are commonly measured in bacterial and mammalian cells. The simplest test systems measure the occurrence of a base-pair substitution mutation in which a single nucleotide is changed followed by a subsequent change in the complementary nucleotide on the other DNA strand. Frame shift mutations occur following the deletion or insertion of one or more nucleotides, which then changes the “reading frame” for the remainder of the gene or multiple genes. Genetic testing for these types of point mutations is generally accomplished by *in vitro* cellular assays for forward or reverse mutations. A forward mutation occurs when there is a detectable change in native DNA whereas a reverse mutation occurs when a mutated cell is returned to its initial phenotype. Both base-pair substitutions and frame shift mutations are routinely measured in bacterial cells by measuring the ability of a cell to acquire the capability to grow in an environment missing an essential amino acid. In these tests, a large number of cells are examined to demonstrate a significant increase in the frequencies of mutations that occur over the frequency of spontaneous mutations.

Chromosomal aberrations are large scale numerical or structural alterations in eukaryotic chromosomes including deletions (visualized as breaks), translocations (exchanges), non-disjunction (aneuploidy), and mitotic recombination. Chromosomal breakage is the classical end point in chromosomal aberration assays. Substances that induce structural changes in chromosomes, especially chromosome breaks, are referred to as "clastogens." To visualize chromosomes and chromosomal aberrations following *in vitro* or *in vivo* treatment with a substance, cells are arrested in metaphase, treated to swell the chromosomes, fixed, transferred to slides and stained. The first metaphase following treatment is the time at which the greatest number of cells with damaged chromosomes may be observed. The most frequently used test systems investigate changes in mammalian cells (such as Chinese hamster ovary or lung cells; human or rat lymphocytes; or human, rat or mouse bone marrow cells) following either *in vitro* or *in vivo* exposure to the test substance. The micronucleus test is a common *in vivo* assay that measures the frequency of micronuclei formation (i.e., chromosomal fragments) in polychromatic erythrocytes.

4.3.3.2 Summary of Mutagenicity Data

A summary of the mutagenicity information for the succinimide dispersants is presented in Table 7. *In vitro* bacterial gene mutation assays and *in vitro* and *in vivo* chromosomal aberration assays have been conducted for both members of the category. Frequencies of reverse mutations in bacteria were not significantly changed after exposure to the succinimide dispersants. *In vitro* and *in vivo* chromosomal aberration studies indicate that succinimide dispersants are not clastogenic.

4.3.3.2.1 Bacterial Gene Mutation Assay

Both substances in this category have been adequately tested in a bacterial reverse mutation test (OECD Guidelines 471 and/or 472). Both tested substances were negative for mutagenic activity, with and without metabolic activation.

4.3.3.2.2 Mammalian Gene Mutation Assay in Transformed Cells

One substance in this category was tested in an *in vitro* mouse lymphoma cell mutagenicity assay (Guideline 476, *In vitro Mammalian Cell Gene Mutation Test*). The result of this study indicates that, in the absence and presence of hepatic microsome activation, succinimide dispersants are not mutagenic or clastogenic.

4.3.3.2.3 *In vivo* Chromosomal Aberration Assays

One of the substances in this category was tested in an *in vivo* chromosomal aberration assay (OECD Guideline 474, *Mammalian Erythrocyte Micronucleus Test*). The test substance was negative for clastogenicity.

4.3.3.3 Data Assessment and Test Plan for Mutagenicity

Both members of the succinimide dispersant category have been tested for mutagenicity in tests for gene mutations and chromosomal aberrations. The assays included point mutations in bacteria, cultured mammalian cells, and *in vivo* chromosomal aberrations in mice. The findings from all studies were negative for mutagenic potential.

Adequate mutagenicity tests exist for both substances in the succinimide dispersant category. Thus, the genetic toxicity of the category has been evaluated adequately with respect to all mutagenic and clastogenic endpoints, and no additional genetic toxicity testing is proposed for the HPV Challenge Program.

4.3.4 Repeated-dose Toxicity of Succinimide Dispersants

4.3.4.1 Repeated-dose Toxicity Test Methodology

Repeated-dose toxicity studies evaluate the systemic effects of repeated exposure to a chemical over a significant period of the life span of an animal (rats, rabbits, or mice). Chronic repeated-dose toxicity studies are concerned with potential adverse effects upon exposure over the greater part of an organism's life span (e.g., one to two years in rodents). Subchronic repeated-dose studies are also concerned with effects caused by exposure for an extended period, but not one that constitutes a significant portion of the expected life span. Subchronic studies are useful in identifying target organ(s), and they can be used in selecting dose levels for longer-term studies. Typically, the exposure regimen in a subchronic study involves daily exposure (at least 5 consecutive days per week) for a period of at least 28 days or up to 90 days (i.e., 4 to 13 weeks). A recovery

period of two to four weeks (generally included in most study designs) following completion of the dosing or exposure period provides information on whether or not the effects seen during the exposure period are reversible upon cessation of treatment. The dose levels evaluated in repeated-dose toxicity studies are notably lower than the relatively high limit doses used in acute toxicity studies. The NOAEL (no observed adverse effect level), usually expressed in mg/kg/day, defines the dose of test material that produces no significant toxicological effects. If the test material produces toxicity at the lowest dose tested (i.e., there is no defined NOAEL), the lowest dose that produced an adverse effect is defined as the LOAEL (lowest observed adverse effect level). While these studies are designed to assess systemic toxicity, the study protocol can be modified to incorporate evaluation of potential adverse reproductive and/or developmental effects.

Reproductive and developmental toxicity studies generate information on the effects of a test substance on male and female reproductive performance such as gonadal function, mating behavior, conception, and development of the conceptus, parturition, and post-partum development of the offspring. Various study designs exist, but they all involve exposure to both male and female test animals before mating. The rat is most often selected as the test species. The test substance is administered to males and females continuously at several graduated doses for at least two weeks prior to mating and until the animals are sacrificed. The males are treated for at least two more weeks. Male gonadal histopathology is carefully assessed at the end of the study. The females are treated through parturition and early lactation. The adult females and offspring are typically studied until termination on post-natal day 21, or sometimes earlier. In addition to providing data on fertility and reproduction, this study design provides information on potential developmental toxicity following prenatal and limited post-natal exposure to the test substance. An NOAEL or LOAEL is also used to describe the results of these tests, with the exception that these values are derived from effects specific to reproduction or development.

The “toxicity to reproduction” requirement in the HPV Challenge Program can be met by conducting the *Reproduction/Developmental Toxicity Screening Test* (OECD Guideline 421) or by adding this screening test to a repeated-dose study (OECD Guideline 422, *Combined Repeated-Dose Toxicity Study with the Reproductive/Developmental Toxicity Screening Test*). The *One-Generation Reproduction Toxicity Study* (OECD Guideline 415) is a more comprehensive protocol for the study of the effect of a test material on reproduction and development that also meets the OECD SIDS and the HPV Challenge Program requirements.

4.3.4.2 Summary of Repeated-Dose Toxicity Data

A summary of the results from the repeated-dose studies for the succinimide dispersant category is presented in Table 8. Repeated-dose toxicity tests have been performed on both members of the succinimide dispersant category by two routes of administration in rats.

4.3.4.2.1 Systemic Toxicity Tests

Both substances in the succinimide dispersant category have been tested for subchronic toxicity.

Mono alkenyl succinimide derivative (CAS # 67762-72-5) was evaluated in a 28-day repeated-dose dermal toxicity study in rats (methodology consistent with OECD

Guideline 410, *Repeated-Dose Dermal Toxicity: 21/28 Day*). The doses used in this study were 10, 40, and 80% solutions of the test material diluted in mineral oil. Twelve male and female rats were included in each test material and vehicle control groups. The test material was applied at a volume of 1 ml/kg to intact skin (clipped of hair), once per day, five days/week for twenty doses. No wrapping was used, but plastic collars were employed to prevent ingestion of the test material. The daily duration of exposure was 6 hours. There were no remarkable findings in this study for any of the endpoints evaluated. A NOAEL for systemic toxicity of ~800 mg/kg/day was established for this study.

Bis alkenyl succinimide derivative (CAS # 84605-20-9) was evaluated in a 28-day repeated-dose oral toxicity study in rats (OECD Guideline 407, *Repeated-Dose 28-Day Oral Toxicity Study in Rodents*). The test material in corn oil was administered to rats by oral gavage at 100, 500, and 1000 mg/kg/day for 28 consecutive days. Control animals received corn oil. There were no remarkable findings in this study for any of the endpoints evaluated. The NOAEL was established at 1000 mg/kg/day.

4.3.4.2.2 Reproduction and Developmental Toxicity Tests

Bis alkenyl succinimide derivative (CAS # 84605-20-9) was tested for reproduction and developmental toxicity (OECD Guideline 421 *Reproduction/ Developmental Screening Test*). The test material in corn oil was administered to rats by oral gavage at doses of 100, 500, and 1000 mg/kg/day. Male and female rats in each dose group received daily treatment for 28 days prior to, and during, the mating period. In addition, the females were treated during gestation and through day 4 of lactation. Control animals received corn oil. Results. There were no remarkable findings in this study for any of the endpoints evaluated. The NOAEL was established at 1000 mg/kg/day.

4.3.4.3 Data Assessment and Test Plan for Repeated-dose Toxicity

Two repeated-dose toxicity studies have been conducted with the two category members. Neither repeated oral administration nor dermal application caused any adverse effects. In addition, one reproductive and developmental toxicity screening study has been conducted on a member of the category. No adverse effects on reproduction or development were observed in this study. The data indicate the members of the succinimide dispersant category are of low concern for repeated-dose toxicity and reproductive and developmental toxicity. Bridging will be used to fill the data gap for the other substance in the category for reproductive and developmental toxicity.

Both substances in this category have been adequately tested for repeated-dose toxicity. By bridging the existing reproductive and developmental toxicity data to the other substance that lacks this data, the reproductive and developmental toxicity of this category has also been adequately evaluated with respect to all endpoints. Therefore, no additional repeated-dose toxicity or reproductive and developmental toxicity testing is proposed for the HPV Challenge Program for this category.

Table 1. Members of the Succinimide Dispersant Category

CAS Number	Chemical Name	**Simplified Chemical Name**
67762-72-5	2,5-Pyrrolidinedione,1-[2-[[2-[[2-[(2-aminoethyl)amino]ethyl]amino]ethyl]amino] ethyl]-, monopolyisobutenyl derivatives	Mono alkenyl succinimide derivative
84605-20-9	Amines, polyethylenepoly-, reaction products with succinic anhydride polyisobutenyl derivatives	Bis alkenyl succinimide derivative

Table 2. Chemical Structures of Succinimide Dispersants

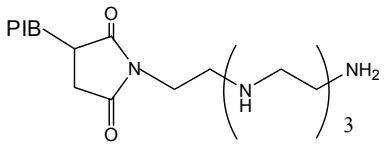
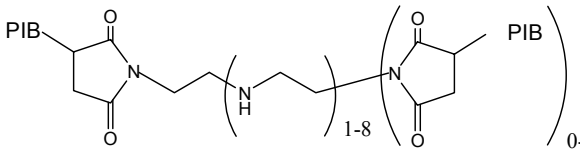
CAS Number	Chemical Structure
67762-72-5	 67762-72-5 PIB= Polyisobutylene 500-2500MW
84605-20-9	 84605-20-9 PIB= Polyisobutylene 500-2500MW

Table 3. Physicochemical Properties of Succinimide Dispersants

CAS Number	Molecular Weight	Specific Gravity[1] g/ml	Viscosity[2] cSt @ 40°C	Melting Point[3] °C	Boiling Point[4] °C	Vapor Pressure[5] Pa	Water Solubility mg/L	Log Kow
67762-72-5	1134	0.9135	1330	NA	841.5	$<1X10^{-10}$	0.125	6.7
84605-20-9	2859-3160	0.9071	1100	NA	1271.5	$<1X10^{-10}$	NDA[6]	NDA[6]

[1]ASTM D1298-99, Standard Test Method for Density, Relative Density (Specific Gravity), or API Gravity of Crude Petroleum and Liquid Petroleum Products by Hydrometer Method
[2]ASTM D 445-97, Standard Test Method for Kinematic Viscosity of Transparent and Opaque Liquids (the Calculation of Dynamic Viscosity)
[3]Not applicable; substances are viscous liquids at ambient tempertures.
[4]Modeling data for theoretical "de-oiled" substance.
[5] Theoretical "de-oiled" succinimide dispersants are solid. Vapor pressure is estimated from the vapor pressure of the petroleum base stock in which the substance is manufactured.
[6]No data needed; bridging from other members of the category.

Table 4. Evaluation of Environmental Fate Information for Succinimide Dispersants

CAS Number	BIODEGRADABILITY	HYDROLYSIS	PHOTODEGRADATION
	Available Data & Proposed Testing	Available Data & Proposed Testing	Available Data & Proposed Testing
67762-72-5	No testing needed Bridging	No testing needed[1]	Direct photodegradation evaluation AOPWIN Model Estimation
84605-20-9	16% biodegraded after 28 days	No testing needed[1]	Direct photodegradation evaluation AOPWIN Model Estimation

[1] Prepare technical discussion.

Table 5. Evaluation of Aquatic Toxicology of Succinimide Dispersants

CAS Number	ACUTE TOXICITY TO FISH 96-hr LL_{50} (mg/L)[1]	ACUTE TOXICITY TO INVERTEBRATES 48-hr EL_{50} (mg/L)[1]	TOXICITY TO ALGAE 96-hr EL_{50} (mg/L)[1]
	Available Data & Proposed Testing	Available Data & Proposed Testing	Available Data & Proposed Testing
67762-72-5	>1,000 (WAF[2], F)	>1,000 (WAF[3], D)	>1,000 (WAF[3], P, R) >1,000 (WAF[3], P, B)
84605-20-9	>1,000 (WAF[2], T)	>1,000 (WAF[3], D)	320 (WAF[3], P, R) 270 (WAF[3], P, B)

[1]Toxicity endpoints are expressed as median lethal loading rates (LL_{50}) for fish and median effective loading rates (EL_{50}) for *Daphnia* and algae. The EL/LL_{50} is defined as the loading rate that adversely effects 50% of the test organisms exposed to it during a specific time. The greater the EL/LL_{50} the lower the toxicity.
[2]WAF = Water accommodated fraction static renewal test.
[3]WAF = Water accommodated fraction static non-renewal test.
F = fathead minnow, *Pimephales promelas*.
D = freshwater cladoceran, *Daphnia magna*.
P = freshwater algae *Pseudokirchneriella subcapitata* formerly called *Selenastrum capricornutum*.
T = rainbow trout, *Oncorhynchus mykiss* formerly called *Salmo gairdneri.*
R = algae growth rate.
B = algae biomass.

Table 6. Evaluation of Acute Mammalian Toxicology of Succinimide Dispersants

CAS Number	ACUTE ORAL TOXICITY[1] Available Data & Proposed Testing	ACUTE DERMAL TOXICITY[1] Available Data & Proposed Testing
67762-72-5	LD_{50} > 5.0 g/kg (rat)	LD_{50} > 5.0 g/kg (rabbit)
84605-20-9	LD_{50} > 5.0 g/kg (rat)	LD_{50} > 2.0 g/kg (rat)

[11]Toxicity endpoints are expressed as median lethal dose (LD_{50}) for acute oral and dermal toxicity.

Table 7. Evaluation of Mutagenicity of Succinimide Dispersants

CAS Number	GENE MUTATION ASSAY Available Data & Proposed Testing	CHROMOSOMAL ABERRATION ASSAY Available Data & Proposed Testing
67762-72-5	Bacterial Reverse Mutation Assay – Not mutagenic	Mouse Lymphoma Mutagenicity Screen – Not clastogenic
84605-20-9	Bacterial Reverse Mutation Assay – Not mutagenic	Mouse Micronucleus Assay – Not clastogenic

Table 8. Evaluation of Repeated-dose Mammalian Toxicology of Succinimide Dispersants

CAS Number	REPEATED-DOSE TOXICITY Available Data & Proposed Testing	REPRODUCTIVE/DEVELOPMENTAL TOXICITY Available Data & Proposed Testing
67762-72-5	28-day repeated-dose dermal study in rats (OECD 410) NOEL = ~800 mg/kg/day (highest dose tested)	No testing needed Bridging
84605-20-9	4-week repeated-dose oral study in rats (OECD 407) NOEL = 1000 mg/kg/day <u>At 1000 mg/kg/day,</u> • No significant effects; <u>At 500 mg/kg/day,</u> • No significant effects; <u>At 100 mg/kg/day,</u> • No significant effects.	Reproduction/developmental oral toxicity screening test in rats (OECD 421) NOEL P0 = 1000 mg/kg/day NOEL F1 = 1000 mg/kg/day <u>At 1000 mg/kg/day,</u> • No significant effects; <u>At 500 mg/kg/day,</u> • No significant effects; <u>At 100 mg/kg/day,</u> • No significant effects.

CAS Number	Environmental Fate					Ecotoxicity			Human Health Effects				
	Physical Chem	Photodeg	Hydrolysis	Fugacity	Biodeg	Acute Fish Toxicity	Acute Invert Toxicity	Algal Toxicity	Acute Toxicity	Point Mutations	Chrom Effects	Sub-chronic	Repro / Develop
67762-72-5	C	C	D	C	B	A	A	A	A	A	A	A	B
84605-20-9	C	C	D	C	A	A	A	A	A	A	A	A	A

A Adequate data available
B Bridging data from anther category member
C Computer modeling
D Technical discussion proposed
T Test

Appendix I. Application of Environmental Fate Modeling to the Succinimide Dispersants Category

This document describes the application and results of computer modeling to estimate the physicochemical properties and environmental distribution of members in a lubricant additives category. Two structures representative of two members of the HERTG (Health, Environmental, and Regulatory Task Group) Category 25, Succinimide Dispersants, where applied to two computer models. The computer model, EPIWIN® (1), was used to estimate the physicochemical properties of the two structures. These calculated properties were used as input data to a Mackay multimedia fate model, which was used to predict their relative environmental distribution.

The US EPA has agreed that computer modeling techniques are an appropriate approach to estimating chemical partitioning and distribution in the environment. Specifically, fugacity based, multimedia fate modeling can be used to compare the relative distribution of chemicals between environmental compartments (i.e., air, soil, water, suspended sediment, sediment, biota). A widely used model for this approach is the EQC model (2). EPA cites the use of this model for this purpose in its document titled *Determining the Adequacy of Existing Data*, which was prepared for the HPV chemical program.

There are three "levels" of the EQC model. In its document, EPA states that it accepts Level I fugacity modeling to estimate transport/distribution values. In the same document EPA states that Level III model data are considered "more realistic and useful for estimating a chemical's fate in the environment on a regional basis". However, the selection and application of any one of the three models should not be done without considering their appropriateness for use with chemical(s) of interest. This includes a basic understanding of selected physicochemical properties of the chemicals to be modeled, as well as the model.

The EQC Level I model requires values for certain basic physicochemical properties of the chemicals to be modeled. The model uses input values including molecular weight, vapor pressure, water solubility, and octanol-water partition coefficient. Another model was needed to estimate these physicochemical properties from the structures of the Category 25 structures. The model used for this purpose was EPIWIN, version 3.04 (1), which is also used by the EPA and which they developed jointly with Syracuse Research Corporation. EPIWIN includes algorithms for estimation of the properties needed for the application of the EQC model.

The succinimide dispersants in Category 25 are related in structure. The structures representing the two CAS numbers in this category are pictured in Table 1. These structures were used to calculate the physical properties, which are shown in Table 2. The data in Table 2 include the basic input values that were used with the EQC model. The results of the EQC model are listed in Table 3.

The structures in Table 1 indicate the position of polyisobutylene (PB) moieties, which have molecular weights of 500 to 2500 daltons. For modeling purposes, physicochemical properties were calculated using PB moieties of 500 daltons. The PB moiety(s) of these substances largely influences the calculated results. The value reported for bioconcentration factor (BCF) does not vary with the molecular weight of the two structures modeled (both give the same value, log BCF of 0.5). This low-modeled value recognizes the low bioaccumulation potential of "super-hydrophobic" substances as a result of low bioavailability.

Environmental distribution modeling was performed using a simple equilibrium distribution model (Mackay Level I Model, version 1.0). The percent distribution results by environmental

compartment are shown in Table 3. These data suggest that succinimide dispersants have the potential to partition primarily to the soil compartment. They have a much lower potential to partition to sediment. These substances are not calculated to partition to the remaining compartments. The two substances in this category can be characterized as having negligible vapor pressure and water solubility, which is why very low percentages of these substances are calculated to partition to the air and water. Additionally, no appreciable transport through the environment is expected as suggested by the fugacity values for these substances (Table 3).

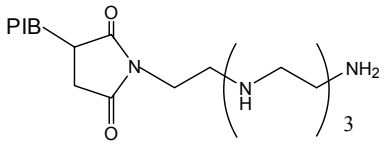
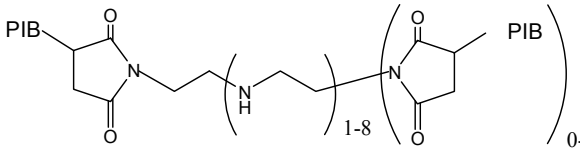
References

1. EPIWIN. 1999. Estimation Program Interface for Windows, version 3.04. Syracuse Research Corporation, Syracuse, NY, USA.

2. Mackay, D., A. Di Guardo, S. Paterson, and C. Cowan. 1996. Evaluating the Environmental fate of a Variety of Types of Chemicals using the EQC Model. Environ. Toxicol. Chem. *15*:1627-1637.

Appendix I. Application of Environmental Fate Modeling to the Succinimide Dispersants Category

Table 1. Chemical Structures of Succinimide Dispersants

CAS Number	Chemical Structure*
67762-72-5	 67762-72-5 PIB= Polyisobutylene 500-2500MW
84605-20-9	 84605-20-9 PIB= Polyisobutylene 500-2500MW

Appendix I. Application of Environmental Fate Modeling to the Succinimide Dispersants Category

Table 2. Physical Properties of Representative Structures from Succinimide Dispersants as Modeled by EPIWIN

CAS Number	Molecular Weight	Log K_{ow}	Water Solubility (mg/L)	Vapor Pressure (Pa)	Log K_{oc}	Log Bioconcentration Factor	Melting Point (^oC)	Boiling Point (^oC)	Atmospheric Oxidation	
									OH^- Rate Constant (cm^3/molec-sec)	Half-life (hrs)
67762-72-5*	776.3	13.8	1.8E-11	6.1E-19	12.0	0.5	349.8	841.5	368.9E-12	0.3
84605-20-9**	1277.2	31.8	3.1E-30	3.3E-31	21.4	0.5	349.8	1271.5	214.8E-12	0.6

* The chemical structure used to model data included a PB moiety (Table1) of approximately 500 daltons or a total carbon number of C48.
** The chemical structure used to model data included PB moieties (Table1) of approximately 500 daltons each or a total carbon number of C48.

Table 3. Environmental Distribution of Representative Structures from Succinimide Dispersants as Modeled by EQC Level I

CAS Number	Air (%)	Water (%)	Soil (%)	Sediment (%)	Suspended Sediment (%)	Biota (%)	Fugacity (μPa)
67762-72-5*	0.00	0.00	97.75	2.17	0.07	0.01	2.8e-16
84605-20-9**	0.00	0.00	97.75	2.17	0.07	0.01	9.4e-28

* The chemical structure used to model data included a PB moiety (Table1) of approximately 500 daltons or a total carbon number of C48.
** The chemical structure used to model data included PB moieties (Table1) of approximately 500 daltons each or a total carbon number of C48.

AR201-14078B

Substance Group:	**Group 25**
Summary Prepared by:	**Petroleum Additives Panel** **Health & Environmental Research Task Group**
Date of last update:	**October 9, 2002**
Contact:	**Sarah Loftus McLallen** **American Chemistry Council** **1300 Wilson Boulevard** **Arlington, VA 22209** **1-703-741-5607 (phone)** **1-703-741-6091 (fax)** **Sarah_Loftus@americanchemistry.com**

1.0 Biodegradation

Robust Summary #: 25-Biodeg-1

Test Substance	
CAS #	84605-20-9
Chemical Name	Bis alkenyl succinimide derivative
Remarks	This substance is referred to as Bis alkenyl succinimide derivative in the HERTG's Test Plan for Succinimide Dispersant Category. For more information on the chemical, see Section 2.0 "Chemical Description of Succinimide Dispersant Category" in HERTG's Test Plan for Succinimide Disperant Category.
Method	
Method/Guideline followed	OECD Method 310B; U.S. EPA Method 796.3260; ASTM D5864-95.
Test Type (aerobic/anaerobic)	Aerobic
GLP (Y/N)	Y
Year (Study Performed)	1996
Contact time (units)	28 days.
Inoculum	Activated sludge supernatant from domestic wastewater treatment plant and soil filtrate.
Remarks for test conditions	Inoculum: Activated sludge from domestic waste water treatment plant was sieved through a 2 mm screen and adjusted to a target solids level of 2500mg/l by diluting with tap water. Adjusted sludge was aerated and homogenized in a blender. The sludge was allowed to settle for 30 mins and the supernatant was decanted and added to the flasks at a concentration of 1% v/v. Concentration of test chemical: Sufficient amount of test material was added to each flask, giving 10 mg C/L in the test flasks. Temp of incubation: 23C ± 3C Dosing procedure: No organic solvents were used to facilitate dissolution of the test material. Test material addition was added directly to the treatment group chamber to achieve the final volume. Test Setup: Total volume of liquid in test chambers was 2 L. The biodegradation test was started by bubbling CO_2 free air through the test media. The CO_2 generated within each test chamber was trapped as $BaCO_3$ in the $Ba(OH)_2$ solution and determined by titrating the remaining $Ba(OH)_2$ with 0.05N standardized HCl. Sampling frequency: CO_2 traps were removed for analysis on Days 0, 2, 5, 7, 10, 14, 17, 20, 23, 28 and 29. On day 28, the test was terminated by the acidification of the test chamber to

	release dissolved CO_2. Controls: Blank and positive controls were included; abiotic and toxicity controls were not. Sodium benzoate was used as the reference substance in the positive controls. Analytical method: $Ba(OH)_2$ ("trap") solutions were used downstream of the test flasks to trap generated CO_2 as $BaCO_3$. The CO_2 produced was determined by titrating the remaining $Ba(OH)_2$ with 0.05N standardized HCl. Method of calculating measured concentrations: N/A Other: The sludge was not exposed to the test substance in the laboratory prior to addition to the test flasks. Twenty ml of the supplemented inoculum was combined with test medium within each 4-L erlenmeyer flask. The solutions were aerated with CO2 free air. Standard plate count was 2.1 x 10^5 CFU/mL.
Results	
Test Substance Degradation, % after time	16 % after 28 days
Kinetic (for sample, positive and negative controls)	Reference (Sodium benzoate): 88%. An average percent biodegradation of 60% was achieved within 5 days, thereby fulfilling the criteria for a valid test reaching the pass level by day 14. Test substance: 16 % (28d)
Breakdown Products (Y/N) If yes describe breakdown products	N
Remarks	
Conclusions	Test substance degraded 16 % in 28 days. The reference substance, sodium benzoate, degraded 88% in the same test period.
Data Quality	(1) Reliable without restrictions.
References	This robust summary was prepared from an unpublished study by an individual member company of the HERTG (the underlying study contains confidential business information).
Other	Dated : 5-9-02

AQUATIC ORGANISMS

2.1 Acute Toxicity to Fish

Robust Summary #: 25-FISH-1

Test Substance	
CAS #	84605-20-9
Chemical Name	Bis alkenyl succinimide derivative
Remarks	This substance is referred to as Bis alkenyl succinimide derivative in the HERTG's Test Plan for Succinimide Dispersant Category. For more information on the chemical, see Section 2.0 "Chemical Description of Succinimide Dispersant Category" in HERTG's Test Plan for Succinimide Dispersant Category.
Method	
Method/Guideline followed	Test protocol followed US EPA Toxic Substances Control Act Test Guidelines, Section 797.1400 (1992), and OECD Guidelines for Testing of Chemicals, #203, Fish Acute Toxicity Test (1992).
Test Type	Static renewal test
GLP (Y/N)	Y
Year (Study Performed)	1997
Species/Strain	Rainbow trout (*Oncorhynchus mykiss*)
Analytical Monitoring	Total organic carbon (TOC) measurements of initial (0-h) test solutions and after one day of test (24-h) before renewal of test solutions. Water samples were passed through 0.45-micron filter prior to TOC analysis using US EPA Method 415.1.
Exposure Period (unit)	96 hours
Statistical methods	Statistical analysis to determine median lethal concentrations used the binomial/nonlinear interpolation method (Stephan, C.E. 1983. Computer Program for the Calculation of LC50 Values. US EPA, Duluth, MN, USA.; personal communication). Nominal concentrations were used for the calculations.
Remarks field for test conditions (fill as applicable)	Test Organisms: Acquired from Mt. Lassen Trout Farms, Red Bluff, CA, USA; fish were juveniles with an average total length of 46.4 mm and an average wet weight of 1.3 g for the control fish at test termination (no range reported); test loading rate = 0.87 g biomass/L; fish received no pretreatment; fish held for a minimum of 14 days before testing; fish were not fed during the test. Test System: Individual water accommodated fractions (WAFs) were prepared for each test level and renewed daily. A measured weight of test material was added to a measured volume of dilution water in a glass vessel and stirred with for 20 hours. Stirring was accomplished using a Teflon coated magnetic stir bar. The vortex of the WAF was approximately 10%. Following the mixing period, the test solutions were allowed to stand for 4 hour before the water phase was removed. To avoid removing non-soluble test material from the surface, a siphon was used to remove the exposure solutions from the mixing vessels. The siphoned aqueous phase (WAF) was then used in the aquatic toxicity test. About 80% of the solution in each test level was renewed daily after 24, 48, and 72 hours. There were three 15-L replicates per

	treatment, 10 fish per replicate (30 fish per treatment). Dilution Water: Filtered well water collected at Marblehead, MA, USA, and adjusted to a hardness of 40-48 mg/L as $CaCO_3$. The water was passed through activated carbon, a particle filter, an ultraviolet sterilizer, and then stored in a polyethylene tank where it was aerated. Light: 16-hours of light per day using cool-white fluorescent lights with an intensity of 4 μEin/sec/ m^2. Test Temperature: 11.6 to 13.1 C. Water Chemistry: Dissolved oxygen ranged from 5.8 - 9.9 mg/L; pH ranged from 7.0 – 7.7; conductivity ranged from 160 - 180 umhos/cm; alkalinity was not reported. Element: Mortality Test Levels: Control and 1,000 mg/L WAF loading rates.
Results	Nominal concentrations: 96-hour LL_{50} (reported as "LC50" in the report) = > 1,000 mg/L
Remarks	Measured concentration: TOC Loading Level (mg/L) — TOC (range :mg/L) Control — 2.8 - 3.0 1,000 — 3.4 - 4.2 Analytical Monitoring: TOC levels were not considered to be indicative of actual test material concentrations and results are therefore based on nominal loading rates. Other: • Lethal concentrations are based on nominal loading rates. • Control response was satisfactory. Test Findings: A thin film of insoluble test material was observed in the 1.000 mg/L loading throughout the test in which 97% of organisms survived. No sublethal effects were noted during the test.
Conclusions	96-h LL_{50} = > 1,000 mg/L
Data Quality	(1) Reliable without restriction
References	This robust summary was prepared from an unpublished study by an individual member company of the HERTG (the underlying study contains confidential business information).
Other	Updated: 5-6-02

Robust Summary #: 25-FISH-2

Test Substance	
CAS #	CAS# 67762-72-5
Chemical Name	2,5 Pyrrolidinedione
Remarks	Test material dosed as received, purity not provided.
Method	
Method/Guideline followed	Test protocol followed US EPA Toxic Substances Control Act Test Guideline #797.1400 (1985/1987/1989), OECD Guideline for Testing of Chemicals #203, Fish Acute Toxicity Test (1984).
Test Type	Static renewal test (Water Accommodated Fraction)
GLP (Y/N)	Y
Year (Study Performed)	1991
Species/Strain	Fathead minnow (*Pimephales promelas*)
Analytical Monitoring	Total organic carbon (TOC) measurements of initial (0-h) test solutions and at 24 hours.
Exposure Period (unit)	96 hours
Statistical methods	Statistical analysis of survival data not warranted because there was no mortality in this study.
Remarks field for test conditions (fill as applicable)	Test Organisms: Acquired from Aquatic Research Organisms, Hampton, New Hampshire, age: juvenile, total length: 38 mm average, wet weight: 0.5 g average (no range reported). Loading: <0.5 g biomass/L, Pretreatment: none, fish held for a minimum of 14 days before testing. No feeding during the test. Test System: Individual test concentrations were prepared by combining the appropriate amount of test substance and dilution water in a glass mixing vessel equipped with a magnetic stirrer and stirring these mixtures for approximately 24 hours, settling the mixtures for approximately 1 hour and siphoning the water phase containing the Water Accommodated Fraction (WAF). Test vessels were 20-liter glass aquaria containing 15 liters of test solution. The solution in each test level was renewed daily after 24, 48, and 72 hours. Two 15-L replicates per treatment, 10 fish per replicate (20 per treatment). Dilution Water: Filtered well water collected at Hampton, New Hampshire and adjusted to the appropriate hardness of 176 mg/L as $CaCO_3$. The water was passed through activated carbon, a particle filter, and then an ultraviolet sterilizer, and then it was stored in a polyethylene tank where it was aerated. Light: 16-h light per day using cool-white fluorescent lights with an intensity of 15 uEin/m^2. Test Temperature: 21.6 to 22.8 C. Water Chemistry: Dissolved oxygen: 6.9 – 8.3 mg/L, pH: 7.0 - 7.8, conductivity: 870 – 900 umhos/cm. Alkalinity not reported. Element: Mortality Test Levels: Control, 100, 300, & 1,000 mg/L WAF loading rates. No undissolved test material was seen on the surface of the test vessels during the entire aquatic toxicity test.

	Test Findings: No mortality or signs of toxicity was observed in all treatments and the control throughout the entire test. Calculation of LL_{50} s: Statistical analysis of survival data not warranted. Analytical Monitoring: TOC levels were between 2.8 - 3.2 mg/L in the control, 3.3 – 4.3 mg/L at 100 mg/L loading, between 3.7 - 4.4 mg/L at 300 mg/L loading and 2.9 – 3.8mg/L at the 1,000 mg/L loading. TOC levels were not considered to be indicative of actual test material concentrations and results are therefore based on nominal loading rates. Reference Substance: No
Results	Nominal concentrations: 96-h LL_{50} >1,000 mg/L. This is equivalent to 96-h LL_0 = 1,000 mg/L (no mortality or toxic signs noted).
Remarks	Measured concentration: n/a Unit: mg/L Statistical results: Statistical analysis of survival data not warranted. Other: • Test results reported in original study as "lethal concentrations" are reported in this summary as "lethal loading", because test results are based on WAF loading rates. • Control response was satisfactory.
Conclusions	No mortality or signs of toxicity were observed in any of the treatments (100, 300, and 1,000 mg/L WAF loading rates) or in the control throughout the entire test.
Data Quality	(1) Reliable without restriction
References	Ward, T.J. (1993) Acute Toxicity of The Water Accommodated Fractions (WAFs) of CMA 610 to The Fathead Minnow, *Pimephales promelas.* T.R. Wilbury Study #9176-CMA/ESI-610.
Other	Updated: 5/31/02

2.2 Acute Toxicity to Aquatic Invertebrates (e.g. Daphnia)

Robust Summary #: 25-DAPH-1

Test Substance	
CAS #	84605-20-9
Chemical Name	Bis alkenyl succinimide derivative
Remarks	This substance is referred to as Bis alkenyl succinimide derivative in the HERTG's Test Plan for Succinimide Dispersant Category. For more information on the chemical, see Section 2.0 "Chemical Description of Succinimide Dispersant Category" in HERTG's Test Plan for Succinimide Dispersant Category.
Method	
Method/Guideline followed	Test protocol followed US EPA Toxic Substances Control Act Test Guideline #797.1300 (1993), OECD Guideline for Testing of Chemicals #202 Daphnia sp. Acute Immobilization Test and Reproduction Test (1984).
Test Type	Static acute toxicity test
GLP (Y/N)	Y
Year (Study Performed)	1996
Species/Strain	Cladoceran, *Daphnia magna*
Analytical Monitoring	Total organic carbon (TOC) measurements were taken of initial (0-h) test solution and at test termination (48-h). Water samples were passed through 0.45 micron filter prior to TOC analysis using US EPA Method 415.1.
Exposure Period (unit)	48 hours
Statistical methods	Statistical analysis to determine median lethal concentrations used the binomial/nonlinear interpolation method (Stephan, C.E. 1983. Computer Program for the Calculation of LC50 Values. US EPA, Duluth, MN, USA.; personal communication). Nominal concentrations were used for the calculations.
Remarks field for test conditions (fill as applicable)	Test species: Juvenile daphnids less than 24-hours old were produced from laboratory in-house culture. Daphnids received no pretreatment; no mortality reported in the culture during the 48 hours prior to test start. Test System: Individual water accommodated fractions (WAFs) were prepared for each test level. Each of the five WAFs was prepared by combining the appropriate amount of test substance and dilution water in a mixing vessels equipped with a magnetic stir bar and stirred for approximately 20 hrs. Mixing speed was adjusted such that a vortex formed that was approximately 25% of the distance to the bottom of the mixing vessel. Following the mixing period, the test solutions were allowed to stand for 4 hrs before the water phase was removed. The water phase (i.e., WAF) was used for the aquatic toxicity test. Test conditions: Two 300 mL glass beakers that contained 250 mL of test solution were used per treatment. Ten Daphnids were used per replicate (20 per treatment).

	Light: 16 hours light and 8 hours dark per day using cool-white fluorescent lights with an intensity of ~6 uEin/m^2sec. Test temperature: 19.4 – 21.0 ºC Dilution water: Laboratory dilution water with a hardness of 160-180 mg/L as $CaCO_3$. Water chemistry: Dissolved oxygen: 7.4 -8.8 mg/L; pH: 7.4–8.8; conductivity: 560-610 umhos/cm. Element: Immobilization. WAF loading rates: Contol, 130, 220, 360, 600, and 1,000 mg/L WAF loading rates. 10 daphnids per replicate (20 per treatment). No undissolved test material was seen on the surface of the test vessels during the entire test.
Results	Nominal concentrations: 48-hr EC_{50} = > 1,000 mg/L. This is equivalent to 48-hr EL_{50} = > 1,000 mg/L based on WAF loading rates.
Remarks	Measured concentration: TOC Loading Level (mg/L) — TOC (range) Control — 3.7 - 3.8 130 — 4.0 - 3.7 1,000 — 4.2 - 4.3 Analytical Monitoring: TOC levels were not considered to be indicative of actual test material concentrations and results are therefore based on nominal loading rates. Other: • Effect concentrations are based on nominal loading rates. • Control response was satisfactory.
Conclusions	48-h EL_{50} = > 1,000 mg/L
Data Quality	(1) Reliable without restriction
References	This robust summary was prepared from an unpublished study by an individual member company of the HERTG (the underlying study contains confidential business information).
Other	Dated: 5-6-02

Robust Summary #: 25-DAPH-2

Test Substance	
CAS #	67762-72-5
Chemical Name	2,5 Pyrrolidinedione
Remarks	Test material dosed as received, purity not provided.
Method	
Method/Guideline followed	Test protocol followed US EPA Toxic Substances Control Act Test Guideline #797.1300 (1985, 1987), OECD Guideline for Testing of Chemicals #202 *Daphnia* sp. Acute Immobilization Test and Reproduction Test (1984).
Test Type	Static acute toxicity test (Water Accommodated Fraction)
GLP (Y/N)	Y
Year (Study Performed)	1991
Species/Strain	Cladoceran, *Daphnia magna*
Analytical Monitoring	Total organic carbon (TOC) measurements of initial (0-h) test solutions and at test termination (48-h).
Exposure Period (unit)	48 hours
Statistical methods	Statistical analysis of data not warranted.
Remarks field for test conditions (fill as applicable)	Test species: Juvenile daphnids less than 24-hours old were produced from laboratory in-house culture. Test System: Individual WAFs were prepared for each test level. A measured weight of test material was added to a measured volume of dilution water in a glass vessel and stirred for 24 hours. Stirring was accomplished using a magnetic stir bar. Following the mixing period, the test solutions were allowed to stand for 1 hour before the water phase was removed. The siphoned water phase (i.e., WAF) was used for the aquatic toxicity test. Test conditions: Two 250-mL glass beakers that contained 200 mL of test solution were used per treatment. Light: 16-hour light per day using cool-white fluorescent lights with an intensity of 9 $uEin^{-1}/m^{-2}$. Test temperature: 19.7 – 20.9°C Dilution water: Filtered well water collected at Hampton, New Hampshire and adjusted to the appropriate hardness 176 mg/L as $CaCO_3$. The water was passed through activated carbon, a particle filter, and then an ultraviolet sterilizer and stored in a polyethylene tank where it was aerated. TOC levels were 2.8-3.9 mg/L at the beginning and 1.7-3.2 mg/L at the end of the test. Water chemistry: Dissolved oxygen: 7.9 – 9.2 mg/L; pH: 7.0 - 8.4; conductivity: 870 – 900 umhos/cm. Element: Immobilization/mortality

	Test Levels: Control, 100, 300, & 1,000 mg/L WAF loading rates: 10 daphnids per replicate (20 per treatment). No undissolved test material was seen on the surface of the test vessels during the entire test. Test Findings: No dead organisms were observed in the control or treatment groups at 24 or 48 hours. At 24 hours and 48-hours 100%, and 40% immobilization respectively were reported for the 1,000 mg/L group. Calculation of EL_{50} s: Statistical analysis of survival data not warranted. Exposure period: 48 hours Analytical Monitoring: TOC levels were 2.8 –1.9mg/L in the control, 2.0 - 3.8 mg/l at 100, 2.2 - 3.7 mg/L at 300 mg/L, and 2.2 - 3.0 mg/L at 1,000 mg/L loading. TOC levels were not considered to be indicative of actual test material concentrations and results are therefore based on nominal loading rates.
Results	Nominal concentrations: 48-h EL_{50} >1,000 mg/L. This is equivalent to 48-h EL_0 = 1000 mg/L based on WAF loading rates.
Remarks	Measured concentration: n/a Unit: mg/L Statistical results: Not applicable. Other: • Control response was satisfactory. • Test results are based on WSF loading rates.
Conclusions	The test material was not toxic to daphnids at loading rates tested. The 48 hour median effective concentration was >1000 mg/L (expressed as the nominal concentration used to prepare the WAF) and the 48-hour no observed effect concentration was 1000 mg/L.
Data Quality	(1) Reliable without restriction
References	Ward, T.J. (1993) Acute Toxicity of the Water Accommodated Fractions (WAFs) of CMA #610 to the Daphnid, *Daphnia magna.* T.R. Wilbury Study #9178-CMA/ESI-610.
Other	Updated: 6/4/02

2.3 Acute Toxicity to Aquatic Plants (e.g. algae)

Robust Summary #: 25-ALG-1

Test Substance	
CAS #	84605-20-9 (1998)
Chemical Name	Bis alkenyl succinimide derivative
Remarks	This substance is referred to as Bis alkenyl succinimide derivative in the HERTG's Test Plan for Succinimide Dispersant Category. For more information on the chemical, see Section 2.0 "Chemical Description of Succinimide Dispersant Category" in HERTG's Test Plan for Succinimide Dispersant Category.
Method	
Method/Guideline followed	Test protocol followed US EPA Toxic Substances Control Act Test Guidelines, Section 797.1050, Algal Acute Toxicity Test (1992), and OECD Guidelines for Testing of Chemicals, Method #201, Algal Growth Inhibition Test (1984).
Test Type	Static Test
GLP (Y/N)	Y
Year (Study Performed)	1998
Species/Strain	Freshwater alga, *Pseudokirchneriella subcapitata* formerly called *Selenastrum capricornutum*
Element basis (# of cells/mL)	10^{+4} cells/mL
Exposure period/duration	96 hours
Analytical monitoring	Total organic carbon (TOC) measurements were taken at test initiation (0-hr) and termination (96-hr) from control, low, and high treatment solutions. Water samples were passed through 0.45-micron filter prior to TOC analysis using EPA Method 415.1
Statistical methods	The probit method (Stephan, C.E., 1983. Computer Program for the Calculation of LC50 Values. US EPA, Duluth, MN, USA.; personal communication) was used to calculate the EC50 values.
Remarks field for test conditions (fill as applicable)	Test Species: Cells taken from an in-house culture of *Pseudokirchneriella subcapitata* that was originally obtained from the University of Texas at Austin alga collection in January 1997. Test System: Individual water accommodated fractions (WAFs) were prepared for each test level. A measured weight of test material was added to a measured volume of dilution water in a glass vessel and stirred with for approximately 24 hours. Stirring was accomplished using a Teflon coated magnetic stir bar. Mixing speed adjusted such that the vortex extended from the surface approximately 5% of the way to the bottom of the mixing vessel. Following the mixing period, the test solutions were allowed to stand for 4 hour before the water phase was removed. The siphoned aqueous phase (WAF) was then used in the aquatic toxicity test. Test Conditions: A static test was conducted; i.e., there was no daily renewal of test solution. There were three 100-mL replicates per treatment loading, each with an inoculum of 10,000 cells/ml. During

	the test all treatment and control flasks were randomly placed on a rotary shaker adjusted to approximately 100 rpm and located in an incubator during the test under constant light (24 hours/day). Daily cell counts were made visually by means of direct microscopic examination with a hemocytometer. Light: Cool-white fluorescent lights provided a light intensity of approximately 380 footcandles. Test temperature: 23.2 to 24.0 C. Dilution Water: Sterile enriched alga growth media (US EPA, 1978, T.R. Wilbury SOP #6) adjusted to pH 7.5. Measured TOC and total suspended solids in fresh untreated alga media were <1.0 to 1.1 and <10 mg/L, respectively at the beginning of the test and 3.1 to 3.6 and 27, respectively at the end of the test. Test Levels: Control, 33, 65, 130, 250, 500, and 1,000 mg/L WAF loading rates. No undissolved test material was observed in any vessels during the test. Calculation of EL_{50} s : The probit method (Stephan, C.E., 1983) was used to calculate the 72, and 96 hour effective concentrations (EC10s, EC50s, and EC90s). Exposure period: 96 hours
Results	Nominal concentrations: 72- and 96-hour ELgr50 = 320 and 510 mg/L, respectively, based on growth rate measurements. 72- and 96-hour ELb50 = 270 and 370 mg/L, respectively, based on biomass measurements.
Remarks	Measured concentration: TOC Analytical Monitoring: TOC (total organic carbon) levels were <1.0 to 1.1 mg/L and 3.1 to 3.6 mg/L in control vessels at test initiation and at 96 hours, and <1.0 to 1.1mg/L and 1.5 to 1.8mg/L in the 1,000 mg/L test vessels at test initiation and at 96 hours. TOC levels were not considered to be indicative of actual test material concentrations and results are therefore based on nominal loading rates. Unit: mg/L Other: • Effect concentrations based on nominal loading rates. • Test results reported in original study as “effect concentrations” (EC) are reported in this summary as “effective loading” (EL), because test results are based on WAF loading rates and not measured concentrations. • Control response was satisfactory.

Conclusions	No effects on cell size, shape, color, adhesion, or aggregation were noted in any of the loading treatments. The test material was algistatic and not algicidal to the freshwater alga at the highest loading rate tested, 1,000 mg/L. This was determined by removing an aliquot of test media from a 96-hour sample, incubating it in fresh medium, and measuring biomass after 4 days incubation.
Data Quality	(1) Reliable without restriction
References	This robust summary was prepared from an unpublished study by an individual member company of the HERTG (the underlying study contains confidential business information).
Other	Updated: 5-8-02

Robust Summary #: 25-ALG-2

Test Substance	
CAS #	67762-72-5
Chemical Name	2,5 Pyrrolidinedione
Remarks	Test material dosed as received, purity not provided.
Method	
Method/Guideline followed	Test protocol followed US EPA Toxic Substances Control Act Test Guideline #797.1050 (1985, 1987), OECD Guideline for Testing of Chemicals #201 Alga, Growth Inhibition Test (1984).
Test Type	Static acute toxicity test
GLP (Y/N)	Y
Year (Study Performed)	1992
Species/Strain	Freshwater algae, *Pseudokirchneriella subcapitata* formerly called *Selenastrum capricornutum*
Element basis (# of cells/mL)	Approximately 10,000 cells/mL
Exposure period/duration	96 hours
Analytical monitoring	Total organic carbon (TOC) measurements of each test concentration and control test solutions at initiation and at test termination (96-h). Water samples were passed through 0.45-micron filter prior to TOC analysis using EPA Method 415.1
Statistical methods	A parametric one-way analysis of variance (ANOVA) and Dunnett's test were used to calculate the no-observed effect level.
Remarks field for test conditions (fill as applicable)	Test Species: Cells taken from a log-growth phase in-house culture of *Pseudokirchneriella subcapitata* that was originally purchased from University of Texas at Austin alga collection. Several range finding studies were conducted prior to the performance of the definitive studies Test System: This study was conducted twice using WAFs prepared under two different sets of experimental conditions. In the first study Individual WAFs were prepared for each test level. A measured weight of test material was added to a measured volume of dilution water in a glass vessel and stirred for 24 hours. Stirring was accomplished using a magnetic stir bar. Mixing speed was adjusted such that a vortex formed between 30 to 50% of the distance to the bottom. Following the mixing period, the test solutions were allowed to stand for 1 hour before the water phase was removed. To avoid removing test material from the surface or bottom, a siphon was placed in the mixing vessel prior to addition of water and test substance with the lower end 1-2 inches off the bottom. The siphoned water phase (i.e., WAF) was used for the aquatic toxicity test. In the second study Individual WAFs were prepared for each test level. A measured weight of test material was added to a measured volume of dilution water in a glass vessel and stirred for 96 hours rather than 24 hours. Stirring was accomplished using a magnetic stir bar. Mixing speed was adjusted such that a vortex formed that extended approximately 5% (rather than 30 to 50%) of the distance to the bottom. Following the mixing period, the test solutions were allowed to stand for 1 hour before the water phase was removed. To

avoid removing test material from the surface or bottom, a siphon was placed in the mixing vessel prior to addition of water and test substance with the lower end 1-2 inches off the bottom. The siphoned water phase (i.e., WAF) was used for the aquatic toxicity test.

At the conclusion of the studies using both the 24 hour WAFs and the 96 hour WAFs, 0.5 ml sub samples of the test media from each flask containing the WAF of the 1000 mg/L solutions of the test substance was combined with 100 ml of fresh media to determine if algicidal or algistatic effects had occurred. These flasks were incubated for 4 days and examined for the presence of algal cells.

Test Conditions: Two static tests were conducted using WAFs prepared under two different sets of experimental conditions [24 hrs (30-50% vortex) and 96 hr (5% vortex) WAFs]. There was no daily renewal of test solution. Three 100-mL replicates per treatment, inoculum ~10,000 cells/mL/test. During the tests all treatment and control flasks were randomly placed on an orbital shaker adjusted to approximately 100 cycles per minute under constant light (24 hours/day). Daily cell counts were made visually by means of direct microscopic examination with a hemocytometer.

Light: Cool-white fluorescent lights provided a light intensity of 50 uEin/m^2sec 24-h per day.

Test temperature:

24 hr WAF Test (30-50% vortex): 23.6 to 24.7^{o}C

96 hr WAF Test (5% vortex): 23.6-24.7 ^{o}C

Dilution Water: Sterile enriched alga growth media (US EPA, 1978, T.R. Wilbury SOP #6) adjusted to pH 7.5. Measured TOC and total suspended solids in fresh untreated alga media were <1.0 and <10 mg/L, respectively. Test media pH was 6.8 – 7.6 at 0-hour and 7.6 - 10.1 after 96 hours.

Test Levels: Control, 1.0, 10, 100, and 1000 mg/L WAF loading rates. During the test with the 24 hrs (30-50% vortex) WAFs, insoluble material was observed on the walls of the 100 and 1000 mg/L WAF test vessel at 24, 48, 72 and 96 hours. During the test with the 96 hr (5% vortex) WAFs, media at all concentration levels were slightly turbid at 24, 48, 72 and 96 hours. All test containers had insoluble material present on the test chamber walls at 24, 48, 72 and 96 hours.

Calculation of EL_{50} s and NOELs: Binomial nonlinear interpolation methods (Stephan, 1983) were used to calculate EC_{50}s (i.e., EL_{50}s). A parametric one-way analysis of variance (ANOVA) and Dunnett's test were used to calculate the no-observed effect level at 72 and 96 hrs.

Method of calculating mean measured concentrations: not applicable.

	Exposure period: 96 hours Analytical monitoring: 24 hr WAF Test (30-50% vortex): At the beginning of the test, TOC measurements were < 1 mg/L in the control and all treated groups, at the end of the test TOC levels were <1 – 1 mg/L in the control and 1 mg/L WAF; and <1 mg/L in all other groups. 96 hr WAF Test (5% vortex): At the beginning of the test, TOC measurements were generally < 1 mg/L in the control, 1, 10, 100 and 1000 mg/L WAF groups. At the end of the test TOC levels were <1 – 1 in control; 1-4 mg/L in the 1 mg/L WAF, 2 mg/L at 10 mg/L WAF, 2-4 mg/L at 100 mg/L WAF and 1-2 mg/l at 1000 mg/L WAF. TOC levels were not considered to be indicative of actual test material concentrations and results are therefore based on nominal loading rates.
Results	24 hr WAF (30-50% vortex): 72-hour 96-hour Based on cell density: (mg/L) (mg/L) EL_{50} 14 21 NOEL 1.0 1.0 Based on growth rate: EL_{50} 24 34 NOEL 1.0 10 96 hr WAF (5% vortex): 72-hour 96-hour Based on cell density: (mg/L) (mg/L) EL_{50} >1000 >1000 NOEL 10 10 Based on growth rate: EL_{50} >1000 >1000 NOEL 10 10 Regrowth of inhibited cultures from both 1000 mg/L test levels [24 hr WAF (30-50% vortex) and 96 hr WAF (5% vortex)] confirmed that the test material was algistatic.
Remarks	Measured concentration: n/a Unit: mg/L Other: • Test results reported in original study as “effect concentrations” and “no observed effect concentrations” are reported in this summary as “effect loading” and “no observed effect levels”, respectively, because test results are based on WAF loading rates. • Control response was satisfactory.

Conclusions	At a loading rate of 1000 mg/L the test material was considered algistatic. The 24 hr WAF (30-50% vortex) had an EL_{50} (72-96 hrs) of 14-34 mg/L and an NOEL of between 1 and 10 mg/L (72-96 hrs). The 96 hr WAF (5% vortex) had an EL_{50} (72-96 hrs) of >1000 mg/L and an NOEL of 10 mg/L (72-96 hrs).
Data Quality	(1) Reliable without restriction
References	Magazu, J.P., (1994) Acute Toxicity of the Water Accommodated Fractions (WAFs) of CMA #610 to the Freshwater Alga, *Selenastrum capricornutum.* T.R. Wilbury Study #73-CM-610. Stephan, C.E. (1983). Computer Program for the Calculation of LC50 Values. U.S. EPA. Duluth, MN. Personal Communication.
Other	Updated: 6/4/02

3.1 Acute Toxicity

3.1.1 Acute Oral Toxicity

Robust Summary #: 25-Acute Oral-1

Test Substance	
CAS #	CAS# 67762-72-5
Chemical Name	2,5 Pyrrolidinedione
Remarks	Test material dosed as received, purity not provided.
Method	
Method/Guideline followed	OECD Guideline 401
Test Type	Acute oral toxicity
GLP (Y/N)	Y
Year (Study Performed)	1985
Species/Strain	Rats/Sprague-Dawley
Sex	Male/Female
No. of animals/dose	5 /sex/group
Vehicle	None
Route of administration	Oral (intragastric)
Dose level	0 and 5000 mg/kg
Dose volume	Not Provided
Control group	Yes
Chemical analysis of dosing solution	No
Remarks field for test conditions	A single dose of the undiluted test material was administered intragastrically to five fasted male and female rats. A control group was included. The animals were observed for signs of toxicity or behavioral changes twice daily. Individual weights were recorded on the day of dosing, on days 2 and 7 and at termination. Body weights of treated animals were compared to control using the Student's t-test. All animals were euthanized, and gross necropsies were performed, at the conclusion of the observation period.
Results	LD50 > 5 g/kg
Remarks	There were no deaths during the study. Diarrhea was observed in two treated males and two treated females on the day of dosing and on the following day. There were no significant differences in mean body weight between the treated and control groups. There were no significant necropsy findings evident in the surviving animals.
Conclusions	The test article, when administered to 5 male and 5 female rats had an acute oral LD50 of > 5 g/kg. No significant toxicity was observed.
Data Quality	Reliable without restriction (Klimisch Code).
References	Unpublished confidential business information
Other	Updated: 8/3/01

Robust Summary #: 25-Acute Oral-2

Test Substance	
CAS #	CAS# 84605-20-9
Chemical Name	Bis alkenyl succinimide derivative
Remarks	Test material dosed as received, purity not provided.
Method	
Method/Guideline followed	OECD Guideline 401
Test Type	Acute oral toxicity
GLP (Y/N)	Y
Year (Study Performed)	1996
Species/Strain	Rats/Crl:CD®(SD)BR
Sex	Male/Female
No. of animals/dose	5 /sex/group
Vehicle	None
Route of administration	Oral (intragastric)
Dose level	5000 mg/kg
Dose volume	5.68 ml/kg of body weight (based on an average bulk density of 0.88 g/ml)
Control group	No
Chemical analysis of dosing solution	No
Remarks field for test conditions	A single dose of the undiluted test material was administered intragastrically to five fasted (17-20 hours) male and female rats. A control group was not included. The animals were observed for signs of toxicity or behavioral changes at 1, 2.5 and 4 hours after dosing and daily thereafter. Mortality checks were conducted twice a day for 13 days and on the morning of day 14. Individual body weights were recorded on the day of dosing, on day 7 and at termination. All animals were euthanized at the conclusion of the observation period. Abbreviated gross necropsy examinations were performed on all animals.
Results	LD50 > 5 g/kg
Remarks	There were no deaths during the study. Dark staining of the anal area was observed in three males on days 1 through 5. All animals were normal by day 6 post dosing. Body weight data were unremarkable. There were no visible lesions observed in any of the animals at necropsy.
Conclusions	The test article, when administered to 5 male and 5 female rats, had an acute oral LD50 of > 5 g/kg. No significant toxicity was observed.
Data Quality	Reliable without restriction (Klimisch Code).
References	Unpublished confidential business information
Other	Updated: 8/3/01

3.1.2 Acute Dermal Toxicity

Robust Summary #: 25-Acute Dermal-1

Test Substance	
CAS #	CAS# 84605-20-9
Chemical Name	Bis alkenyl succinimide derivative
Remarks	Test material dosed as received, purity not provided.
Method	
Method/Guideline followed	OECD Guideline 401
Test Type	Acute oral toxicity
GLP (Y/N)	Y
Year (Study Performed)	1996
Species/Strain	Rats/Crl:CD®(SD)BR
Sex	Male/Female
No. of animals/dose	5 /sex/group
Vehicle	None
Route of administration	Oral (intragastric)
Dose level	5000 mg/kg
Dose volume	5.68 ml/kg of body weight (based on an average bulk density of 0.88 g/ml)
Control group	No
Chemical analysis of dosing solution	No
Remarks field for test conditions	A single dose of the undiluted test material was administered intragastrically to five fasted (17-20 hours) male and female rats. A control group was not included. The animals were observed for signs of toxicity or behavioral changes at 1, 2.5 and 4 hours after dosing and daily thereafter. Mortality checks were conducted twice a day for 13 days and on the morning of day 14. Individual body weights were recorded on the day of dosing, on day 7 and at termination. All animals were euthanized at the conclusion of the observation period. Abbreviated gross necropsy examinations were performed on all animals.
Results	LD50 > 2 g/kg
Remarks	There were no deaths during the study. Dark staining of the anal area was observed in three males on days 1 through 5. All animals were normal by day 6 post dosing. Body weight data were unremarkable. There were no visible lesions observed in any of the animals at necropsy.
Conclusions	The test article, when administered to 5 male and 5 female rats, had an acute oral LD50 of > 2 g/kg. No significant toxicity was observed.
Data Quality	Reliable without restriction (Klimisch Code).
References	Unpublished confidential business information
Other	Updated: 8/3/01

Robust Summary #: 25-Acute Dermal-2

Test Substance	
CAS #	CAS# 67762-72-5
Chemical Name	2,5 Pyrrolidinedione
Remarks	Test material dosed as received, purity not provided.
Method	
Method/Guideline followed	OECD Guideline 402
Test Type	Acute dermal toxicity (Limit Test)
GLP (Y/N)	Y
Year (Study Performed)	1986
Species/Strain	Rabbits/New Zealand White
Sex	Male and female
No. of animals/sex	5
Vehicle	None
Route of administration	Dermal
Dose level	0 and 5 g/kg
Control group included	Yes
Remarks field for test conditions	On the day prior to the initiation of dosing the trunk of each animal was clipped of hair. Animals were reclipped as needed. A single dose of 5 g/kg of the undiluted test material was administered dermally to five male and female rabbits. The test material was kept in contact with the skin for a period of 24 consecutive hours under a plastic sheet that was over wrapped with paper towels to prevent tearing. At the end of the 24-hour exposure period, the application site was wiped clean of residual test material with mineral oil. Collars were placed on all control and treated animals for 24 hours to prevent oral ingestion of residual test material. The animals were observed for abnormal clinical signs frequently after dosing and twice daily for the 14-day study period (once daily on weekends). Dermal examinations were performed on days 1, 7 and 14 according to the modified Draize method. Individual body weights were recorded on day 1, prior to dosing, and on day 2, 7, and 14. The surviving animals were euthanized at the conclusion of the observation period. Gross necropsies were performed on all animals. Sections of skin and any abnormal tissues were examined microscopically.
Results	LD50 > 5.0 g/kg (males and females)
Remarks	No mortality was observed. Slight to well-defined erythema was observed in the treated skin of males and females between days 1 and 7. Slight erythema was observed in the treated skin of all animals one hour after exposure to 5 g/kg of the test material. Three treated females still had well defined erythema six days later, but all had normal skin by day 14. Dermal postulates or abscesses developed in two treated males and one control female. These lesions were observed in the treated area and on the lip between days 7 and 14. Reddened depilitated or flaky skin was observed at previously abscessed sites at necropsy on Day 14. The dermal lesions observed in treated males appeared histologically as trace or moderate hyperkeratosis, mild dermatitis and mild acanthosis. The skin of all treated females was histologically normal. The mean body weight of the treated females was 5% lower than control at study termination.

Conclusions	The test article, when administered dermally as received to 5 male and 5 female Sprague-Dawley rats, had an acute dermal LD50 of greater than 5.0 g/kg. Significant dermal findings were observed in the treated males and females during the first week of study and in the males only at study termination.
Data Quality	Reliable with restriction (Klimisch Code). Restriction due to the presentation of only summary dermal findings in the final report.
References	Unpublished confidential business information
Other	Updated: 8/3/01

3.2 Repeated Dose Toxicity

Robust Summary #: 25- Repeated Tox-1

Test Substance	
CAS #	CAS# 67762-72-5
Chemical Name	2,5 Pyrrolidinedione
Remarks	Test material purity not provided.
Method	
Method/Guideline followed	OECD 410
Test Type	28-day dermal toxicity study in rats
GLP (Y/N)	Y
Year (Study Performed)	1985
Species	Rat
Strain	Sprague-Dawley CD, 10-11 weeks of age at initiation of treatment
Route of administration	Dermal, 6 hour/day, to the clipped, unabraided, dorsal surface.
Duration of test	28 days of treatment (Monday through Friday; 28 total doses administered/animal)
Doses/concentration levels	0, 10, 40, and 80% in mineral oil
Vehicle	Mineral Oil
Sex	Males and females
Dose volume	1 ml/kg
Frequency of treatment	Once/day, 5 days/week
Control and treatment groups	12 animals/sex/group. The control animals were administered the vehicle.
Post exposure recovery period	None
Dose range finding study	Yes
Statistical methods	Body weight, body weight gains, food consumption, hematology and clinical chemistry parameters, organ weights and organ/body weight ratios were analyzed. Mean values of all dose groups were compared to control at each time interval. Tests included parametric ANOVA with a Dunnett's test.
Remarks field for test conditions	The test material was applied to the clipped, unabraided dorsal surface of the rats for 6 hours/day, 5 days/week for 28 days. On each dosing day the appropriate dosing suspension was applied to either the anterior or posterior half of the shaved area. Application sites were alternated on each day of dosing. Animal fur was reclipped twice/week. A plastic collar was placed around each animal's neck to prevent ingestion of the test material. Following the 6-hour exposure the collars were removed and the application site was wiped with gauze moistened with mineral oil. (OECD Guideline 410 suggests the use of a gauze patch over the treatment site secured to the trunk with non-irritating tape and wrapped with an elastic sleeve. This procedure was not used during this study. This is considered a minor deviation from the suggested Guideline.) Animals were examined for viability and signs of toxicity daily. Detailed clinical observations were made weekly. Pupil response was evaluated pretest, on the first and last day of treatment and weekly during the study. Dermal responses were evaluated (Draize) on the

	first and last day of treatment and weekly, on Fridays, during the study. Body weights were recorded twice weekly during the study. Food consumption was recorded weekly during the study. Hematology and clinical chemistry parameters were evaluated for 10 animals/sex/group at termination. Macroscopic examinations were performed on all animals. Select organs were weighed. The lungs, liver, spleen, brain, kidneys, testes, ovaries, skin (treated and untreated) and gross lesions were examined microscopically.
Results	
Remarks	No deaths occurred and no compound related signs of toxicity were observed during the study. The physical observations that were observed included ocular and nasal discharges and alopecia on the forepaws and scabs and sores on the neck. These findings were attributed to the use of collars during treatment and were not considered compound related. Normal pupil responses were observed in all animals throughout the study. Slight to well defined erythema with no or slight edema was seen in both sexes of the treated and control groups. No dose related trends were evident in the incidence or severity of skin irritation at any evaluation interval. These findings were attributed to a vehicle effect. Dry, flaky, and/or abraded skin was observed sporadically in all but the high dose group and was not dose related. Body weights and body weight gain were unremarkable during the study. The mean food consumption value of the mid dose males was slightly (statistically significantly) elevated compared to control during the second week of study. This difference actually reflected a slight decrease in control food consumption and was not attributed to exposure to the test material. Food consumption data was unremarkable in all groups at the remaining evaluation intervals. There were no treatment-related differences from control observed in the hematology data of the treated animals following the treatment period. Serum chemistry values were considered unremarkable in the treated animals at termination. There were several, non-treatment related, statistically significant differences observed in several serum chemistry parameters. These included decreases in mean glucose (mid-dose males) and sodium (males), increased direct billirubin (high-dose males) and uric acid in the low dose females. No dose related trends were evident in these data and all of these findings were within the range of corresponding historical control data. These differences were not considered treatment related. There were no alterations in organ weights that were attributed to treatment with the test material. The mean brain weight of the low dose males was slightly (statistically significantly) lower than control. However, the corresponding brain to body weight ratios were comparable indicating that the decrease observed in mean absolute weights was not treatment related. Several gross pathological observations appeared sporadically and exhibited no dose related trends. They were not considered treatment

	related. Findings included red, thickened or scabbed skin in four control males, dilated renal pelvis in three low dose males, one mid dose female and one high dose female, a fluid filled kidney in one low dose male, red salivary nodes in one control female and mottling or multiple red/purple foci on the thymus in one control and one mid dose male. Red foci in the lungs were seen in one mid dose male and a diaphragmatic hernia was noted in one mid dose female. These findings were not considered treatment related. Microscopic examination of treated skin sites showed acanthosis in both high dose and control animals. Necrosis and ulceration of treated skin was observed only in the controls. There was no increase in the incidence or severity of skin lesions in the high dose animals compared to the controls. The observed dermal changes were attributed to exposure to the vehicle and not to treatment with the test material. Other histopathological changes that were observed were spontaneous or naturally occurring lesions in rats of this strain.
Conclusions	The Study Director concluded that the repeated dermal application of this test material caused no observable signs of toxicity. The No Observed Effect Level was 80% in mineral oil.
Data Quality	Reliable without restriction (Klimisch Code)
References	Unpublished confidential business information
Other	Updated: 8/6/00

Robust Summary #: 25-Repeated Tox-2

Test Substance	
CAS #	CAS# 84605-20-9
Chemical Name	Bis alkenyl succinimide derivative
Remarks	Test material purity not provided.
Method	
Method/Guideline	OECD Guidelines 421 and 407, EPA Guideline 81-8, 82-7, 83-1
Test Type	Combined four week repeated dose oral toxicity, reproduction and neurotoxicity screen in rats
GLP (Y/N)	Y
Year (Study Performed)	1997
Species	Rat
Strain	Sprague-Dawley CD, 51 days of age at initiation of treatment
Route of administration	Orally by gastric intubation
Duration of test	28 Day toxicity phase: 29 or 30 days Reproduction phase: F0 males: 29 day premating period plus mating and postmating periods (70 days total). F0 female: 29 day premating period through day 4 of lactation (54-68 days total). Neurotoxicity phase: 29 days
Dose levels	28 Day toxicity phase: 0, 100, 500 and 1000 mg/kg/day Reproduction phase: 0, 100, 500 and 1000 mg/kg/day Neurotoxicity phase: 0, 1000 mg/kg/day
Vehicle control	Corn Oil
Dose volume	5 mL/kg
Sex	Males and females
Frequency of treatment	Once/day, 7 days/week
Analytical confirmation of concentration.	Homogeneity, stability and weekly dose concentration confirmation.
Control and treatment groups	28-Day toxicity phase: 6/sex/group in all groups plus 6/sex recovery in control and high dose groups. Reproduction phase: 12/sex/group Neurotoxicity phase: 6/sex/group in the control and high dose plus 6/sex recovery in control and high dose groups.
Post exposure recovery period	28 Day toxicity phase: 2 weeks Neurotoxicity phase: 2 weeks
Mating ratio	One male to one female
Duration of mating period	Up to 9 days with initial male; if positive evidence of mating not present (sperm or copulatory plug) then female paired with a second proven breeder male from the same dose group for up to six additional days.
Statistical methods	Anova, Bartlett's test, Dunnett's, Kruskal-Wallis, Dunn's Ranked Sum, Jonckheere's test; F test, Welch's test, t-test, Fisher's Exact Test with Bonferonni correction.
Dose range finding study	3/sex/group at dose levels of 0, 100, 500 and 1000 mg/kg/day treated for 7 days.

Remarks field for test conditions	*Range-Finding, 28 Day Toxicity, Neurotoxicity and Reproduction Study* Viability and Toxicity: twice daily, at least 4 hours apart. Clinical Observations: pretest and weekly throughout the study. Body Weights: pretest, day 1 of treatment, weekly through termination. F0 males: pretest, weekly during premating, mating and post mating periods and at termination. F0 females: pretest, weekly during premating and mating; gestation days 0, 7, 14 and 20; females with litters weighed on lactation days 0 and 4. Unmated females were weighed weekly. Food Consumption: Pretest and weekly during treatment and recovery periods. F0 males: pretest, weekly throughout the study except during mating. F0 females: pretest, weekly during premating, days 0-7, 7-14 and 14-20 of gestation and days 0-4 of lactation for females with litters. Necropsy: 3/sex/group. Macroscopic Examinations: Macroscopic examinations performed on all animals. 28 Day Toxicity Study Phase Hematology, Clinical Chemistry and Urinalysis: 6/sex/group all groups at termination of treatment, 6/sex from the control and high dose group at completion of the two-week recovery period. **Necropsy: Treatment termination-6/sex/group, macroscopic examination. Recovery termination-6/sex/Control and High Dose, macroscopic examination.** Macroscopic Examinations: Macroscopic examinations performed on all animals. Selected organs weighed for all animals. A range of tissues was preserved for all animals in the control and high dose groups. Microscopic Examinations: Gross lesions and tissue masses were examined microscopically for all animals. Neurotoxicity Study Phase Functional Observational Battery (FOB): 12/sex from the control and high dose group pretest and on days 14 and 29 and 6/sex from the control and high dose on day 42. All animals were tested prior to dosing. Animal groups were blind to the evaluator. The FOB included an evaluation of posture, vocalization and palpebral closure, reactivity to general stimuli, assessment of signs of autonomic function including lacrimation, salivation, fur appearance, deposits around the eyes, arousal level and gait, urination and defecation counts, convulsions, tremors, abnormal movements or behaviors, excessive or repetitive actions, piloerection and exophthalmos, approach response, auditory stimuli, tail pinch response, papillary function, hindlimb extensor strength, grip resistance, landing foot splay, air righting ability and body weight. Necropsy: Treatment termination-6/sex/Control and High Dose, macroscopic examination (Day 30). Recovery termination-6/sex/Control and High Dose macroscopic examination (Day 46). Macroscopic Examinations: All males in the control and high dose were perfused for better fixation of the CNS and peripheral nervous tissues. Brain weight and size recorded. The brain and spinal cord

	were sampled for neuropathological evaluation. Tissues sampled included: brain (forebrain, center of cerebrum, midbrain, cerebellum, pons, medulla oblongata), spinal cord (at cervical swellings C3-C7 and at lumbar swellings T13-L4), Gasserian ganglion, Lumbar dorsal route ganglion (T13-L4), Lumbar dorsal root fibers (T13-L4), Lumbar ventral root fibers (T13-L4), Cervical dorsal route ganglion (C3-C7), Cervical dorsal root fibers (C3-C7), Cervical ventral root fibers (C3-C7), Sciatic nerve (mid thigh region), Sciatic nerve (at sciatic notch), Sural, Tibial, Peroneal nerves, Optic nerve and eyes. Microscopic Examinations: The tissues outlined above and all gross lesions and tissue masses were examined microscopically for all neurotoxicity animals. Reproductive Study Phase Pup/Litter Examinations: Litters observed as soon as possible after delivery for number of live and dead pups and pup abnormalities. Thereafter litters observed twice daily for dead pups and/or obvious irregularities through day 4 of lactation. Litter Size: Number of live and dead pups recorded on days 0 and 4 of lactation. Individual Pup Body Weights: Pup weights recorded on days 0 and 4 of lactation. Pup Sex Distribution: Number of male and female pups in each litter recorded on days 0 and 4 of lactation. Necropsy: F0 males Day 71(2 days after the last litter reached lactation Day 4); F0 females with litters sacrificed on lactation Day 4; Unmated females sacrificed 25 days after completion of mating period; Mated females that did not deliver sacrificed 25 days after completion of mating period; F1 pups were sacrificed on lactation Day 4. Macroscopic Examinations: Macroscopic examinations performed on all F0 animals. F1 pups were examined externally and internally including internal sex verification. Tissues retained for possible skeletal evaluation. Selected organs weighed for all animals. A range of tissues was preserved for all animals in the control and high dose groups. Microscopic Examinations: Select tissues examined microscopically for all control and high dose F0 animals. Gross lesions and tissue masses were examined microscopically for all F0 animals.

Results	*Dose Range-Finding Study* No effects of the test material were observed during the dose range finding study on animal survival, clinical observations, body weight and body weight change, food consumption and feed efficiency or postmortem data. The No Observed Effect Level for the dose range-finding study was 1000 mg/kg/day. ***28 Day Toxicity and Neurotoxicity Study Phases*** All animals survived to their scheduled sacrifice. Clinical observations were unremarkable. Body weight and body weight change data were unremarkable in all groups during treatment and recovery. Food consumption data at 100 and 500 mg/kg/day were comparable to control during treatment and recovery. In the 1000 mg/kg/day group food consumption was significantly higher than control during treatment and continued to be elevated in the females during recovery. Feed efficiency in this group was generally comparable to control; however values were lower than control in the 1000 mg/kg/day males at week 4 and during the first week of recovery in the females. Due to the lack of a consistent trend in these data the observed differences from control were not considered by the Study Director, or by this reviewer, to be of toxicological significance. No effects of treatment were observed in the functional observational battery data of the 1000 mg/kg/day group. Landing foot splay distances in the 1000 mg/kg/day group were slightly shorter than control during treatment and recovery. However these data were similar to pretest values. These values did not change during treatment. Neurological insults are usually associated with a lengthening of this distance. This finding was not considered toxicologically significant or treatment related. No treatment related effects were evident in the hematology, clinical chemistry, urinalysis, organ weight, brain size or macroscopic data of the treated animals in the 28-day toxicity or neurotoxicity study phases. The microscopic examination of gross lesions did not indicate any treatment related microscopic findings in the 28-day toxicity study phase. In the neurotoxicity study phase all tissues were within normal limits when examined by light microscopy. The No Observed Effect Level for the 28-day toxicity and neurotoxicity study phases was 1000 mg/kg/day. *Reproductive Study Phase* *F0 (Parental Generation)* All control and treated animals survived to their scheduled sacrifice. Clinical findings were unremarkable. Mean body weight, body weight change, food consumption and feed efficiency were generally unremarkable during the premating period. In some instances body weight gain and food consumption values were slightly elevated compared to control. These differences from control were not considered toxicologically significant. Mean male body weights of the treated animals were generally considered comparable to control during mating and post mating. Some mid and high dose animals

exhibited slight weight loss during the last two weeks of the study. The cause of this finding was unclear. However the lack of any body weight effects during the first eight weeks of study and the lack of any effect on body weight gain suggests that this finding was not treatment related. Postmating period food consumption for the treated males was higher than control (frequently statistically significantly) over the postmating period. This increase in food consumption was not considered an adverse effect or toxicologically significant. Feed efficiency was reduced in the 500 and 1000 mg/kg/day males during the last two weeks of study. These decreases coincided with the reduced body weights observed in some animals in these groups.

Female mating indices for the treated groups were comparable to control. Male mating indices were comparable to control (100%) at 100 (100%) and 500 (100%) mg/kg/day and slightly lower than control at 1000 mg/kg/day (83.3%, 10/12). The lower value in the high dose group was not statistically different from control and was within the range of recent historical control data for the laboratory. It was not considered treatment related.

Mean maternal body weights during gestation were unaffected by treatment. Body weight gain over days 7-14 of gestation in the mid and high dose groups was significantly higher than control. This was not considered treatment related or toxicologically significant. Body weights and gains during lactation were comparable to control. Maternal food consumption during gestation and lactation were comparable to control.

No effects of treatment were seen in the parturition data (number of stillborn pups, number of pups dying between birth and lactation day 4, gestation index, duration of gestation, females completing delivery and mean number of live pups/litter).

The absolute organ weights and organ to body and organ to brain weight ratios of the parental males and females were unremarkable. No treatment effects were evident. There were no treatment related macroscopic or microscopic abnormalities observed in the reproductive study phase. The mean numbers of uterine implantation scars and corpora lutea were comparable between the control and treated groups.

<u>Litter Data</u>
Pup body weights, pup viability indices, and sex ratios were unremarkable. No treatment related effects were evident. There was a slight increase in the number of female pups in the mid dose group at birth. However, in the absence of a similar finding in the high dose, this was not considered a treatment effect. No malformations were seen in stillborn pups or in dead pups found during days 0-4 of lactation in the control or treated groups. There were no macroscopic findings in the pups that were considered related to test material administration.

The No Observed Effect Level for the reproductive study phase was 1000 mg/kg/day.

	The No Observed Effect Level for the overall study was 1000 mg/kg/day.
Remarks	The No Observed Effect Level (NOEL) in this combined 28-day repeated dose oral toxicity, neurotoxicity and reproductive screening study in rats was 1000 mg/kg/day.
Conclusions	This test material did not exhibit any evidence of toxicity when evaluated in a combined 28-day repeated dose oral toxicity, neurotoxicity and reproductive screening study in rats. The NOEL was 1000 mg/kg/day.
Data Quality	Reliable without restriction (Klimisch Code)
References	Unpublished confidential business information
Other	Updated: 8/9/01

3.3 Genetic Toxicity

Robust Summary #: 25-GenTox-1

Test Substance	
CAS #	CAS# 67762-72-5
Chemical Name	2,5 Pyrrolidinedione
Remarks	Test material purity not provided.
Method	
Method/Guideline followed	OECD Guideline 471
Test Type	Bacterial Reverse Mutation Assay
GLP (Y/N)	Y
Year (Study Performed)	1986
Test System	*Salmonella typhimurium*
Strains Tested	*Salmonella typhimurium* tester strains TA98, TA100, TA1535 and TA1537
Exposure Method	Plate incorporation
Test Substance Doses/concentration levels	0.0333, 0.1, 0.3333, 1.0 and 3.333 mg/plate
Metabolic Activation	With and without 400 ul/plate S9 fraction mix of livers of Aroclor 1254 pretreated Sprague Dawley rats)
Vehicle	Tetrahydrofuran diluted 1:10 with dimethylsulfoxide (DMSO)
Tester strain, activation status, Positive Controls and concentration level	TA98 +S9 2-aminoanthracene 2.0 ug/plate TA98 -S9 2-nitroflourene 10.0 ug/plate TA100 +S9 2-aminoanthracene 2.0 ug/plate TA100 -S9 sodium azide 1.0 ug/plate TA1535 +S9 2-aminoanthracene 2.0 ug/plate TA1535 -S9 sodium azide 1.0 ug/plate TA1537 +S9 perylene 30.0 ug/plate TA1537 -S9 9-aminoacridine 50.0 ug/plate
Vehicle Control	Tetrahydrofuran diluted 1:10 with dimethylsulfoxide (DMSO)
Statistical Analysis	Mean revertant colony count and standard deviation were determined for each dose point.
Dose Rangefinding Study	Conducted using tester strain TA100 at dose levels of test material ranging from 0.003 to 3.3 mg/plate without S9.
S9 Optimization Study	Conducted using tester strains TA98 and TA100, and a dose level of test material of 3.3 mg/plate and concentrations of S9 mix ranging from 25 to 400 ul S-9/plate.
Remarks field for test conditions	This study was conducted according to OECD Guideline 471 (1983). Revisions to this Guideline in 1997 suggest the addition of the *E. coli* WP2 uvrA or *S. typhimurium* TA 102 tester strains. Since this study was conducted prior to this revision, these strains were not included. In the main study there were two treatment sets for each tester strain, with (+S9) and without (-S9) metabolic activation. Each of the tester strains was dosed with five concentrations of test substance, vehicle controls, and a positive control. Three plates/dose group/strain/treatment set were evaluated. 0.1 ml of test material, positive control or vehicle control were added to each plate along with 0.1 ml of tester strain, S9 mix (if needed) and 2.5 ml of top agar. This was overlaid onto the surface of 25 ml minimal bottom agar in a petri dish. Plates were incubated for 48 hours at 37^{o}C. The condition of the

	bacterial background lawn was evaluated for cytotoxicity and test article precipitate. An Artek 880 Automatic Colony Counter System was used to count all plates of strains TA1535 and 1537 containing >75 revertant colonies and all plates of strains TA98 and 100 unless precluded by compounding factors (precipitate, agar bubbles). A Quebec Darkfield Counter was used for hand counting all other plates. In order for the test material to be considered positive, two consecutive dose levels (or the highest non-toxic dose level) must produce at least twice (2.5 fold for TA1535, 1537 and 1538) the mean number of revertant counts of the negative/solvent control and these consecutive dose levels must demonstrate a dose response relationship.
Results	The test substance was not mutagenic in this assay with or without metabolic activation.
Remarks	No cytotoxicity was observed in the dose rangefinding study with tester strain TA100 with or without metabolic activation. The S9 optimization study was performed using TA98 and TA100 at 3.3 mg/plate and concentrations of S9 mix of 25-400 ul. In the absence of any effect 400 ul S9 mix/plate was used in the mutagenicity study. In the main study the test material was completely miscible with tetrahydrofuran and partially miscible in subsequent dilutions with DMSO, but was not completely miscible with the top agar at $\geq$0.03 mg/plate. The test material was not cytotoxic to any strain. nor mutagenic to any strain. No reproducible increases in mutation frequency were observed in any tester strain with or without metabolic activation. The tester strains responded to the positive controls as expected.
Conclusions	Under the conditions of this study, the test material was not mutagenic with or without metabolic activation.
Data Quality	Reliable without restriction (Klimisch Code)
References	Unpublished confidential business information
Other	Updated: 8/7/01

Robust Summary #: 25-GenTox-2

Test Substance	
CAS #	67762-72-5
Chemical Name	2,5 Pyrrolidinedione
Remarks	Test material purity not provided.
Method	
Method/Guideline followed	Consistent With OECD Guideline 476
Test Type	Mouse Lymphoma Mutagenicity Screen
GLP (Y/N)	Y
Year (Study Performed)	1986
Test System	L5178Y mouse lymphoma cells Clone 3.7.2C
Culture Preparation and Maintenance	Cell stocks were stored frozen in liquid nitrogen. Cultures were grown in Fisher's medium in a shaker incubator at 125 rpm and 37°C in humidified 5% CO_2 in air and maintained with cell density adjustments at least twice a week for approximately four months. Each time a culture was used it was checked for bacterial or fungal contamination. Prior to use in the assay, cells were treated with methotrexate to reduce the frequency of spontaneously occurring TK-/- cells.
Exposure Method	Dilution
Test Substance Doses/concentration levels	**With metabolic activation: 0.0, 333, 667, 1000, 3330, 6670 ug/ml.** Without metabolic activation Assay A: 0.0, 333, 667, 1000, ug/ml. Without metabolic activation Assay B: 0.0, 500, 1000, 1333, 1667, 2000 ug/ml.
Metabolic Activation	Aroclor 1242/1254 induced rat liver
Vehicle	5% Pluronic F-68 (w/w in distilled water)
Positive Control concentration levels by activation status	With activation: 7,12-dimethylbenzanthracene (DMBA) 5 ug/mL Without activation: ethylmethanesulfonate (EMS) 744 ug/mL
Statistical Analysis	Means and standard deviations were determined. Plates were scored for total number of colonies/plate. Counts were made using an automatic colony counter. Mutation frequency was determined by dividing the average number of colonies in the treated plates by the average number of colonies (x 10^6) in the corresponding vehicle control plates and multiplying by two hundred. By comparing the mutation frequency of the treated plates to that of the control plates, the presence of a significant level of mutagenic activity can be detected.
Test Substance Solubility	Test substance solubility in the vehicle was determined prior to the initiation of the mutagenicity assay.
Toxicity Determination	A preliminary toxicity test with and without S-9 activation was conducted at dose levels ranging from 1 to 5000 ug/ml. The test material was added to each culture tube. After 4 hours the cells were washed and placed into culture for 2 days at 37°C. Test material toxicity was determined by comparing cell population growth at each dose level with that of the solvent controls. Cell population density was determined 24 and 48 hours after the initial exposure to the test material. At 24 hours cell population density was adjusted to 0.3 x 10^6 Test material toxicity was determined by comparing the total suspension growth of the treated cultures at each dose level with that of the average total suspension growth of the negative (solvent) controls. Cell counts were determined using an electronic cell counter.
Mutagenicity Assay (Remarks field for test	This study was conducted prior to the development of OECD Test Guideline 476. This study deviates from this guideline in that colony sizing was not

conditions)	performed. This deviation from the guideline was not considered sufficient to invalidate this study. Each test material concentration and the positive controls were tested in singlet. Negative controls were tested in duplicate. Based on the toxicity determination the test material was prepared so that the highest concentration would yield a percent total growth of $\geq$10%. The test material was solubilized and serial dilutions were carried out in order to yield approximately 90% total growth at the lowest dose level. The test material was added to cells, with and without activation, placed on a roller drum at 25 rpm at 37^{o}C for 4 hours. Cells were then washed and placed in suspension cultures at a cell density of 0.3 x 10^6 cells/ml. In the mutagenicity study there were two treatment sets for each concentration of test substance, with (+S9) and without (-S9) metabolic activation. DMBA (positive control) was tested with activation and EMS (positive control) was tested without activation. The washed cells were replaced on the mixer at 37^{O}C for two days. Cell population adjustments were made at 24 hours to yield a cell population of 0.3 x 10^6 cells/ml. The cells were then plated in a restrictive media containing trifluorothymidine (TFT) which allows only the $TK^{-/-}$ cells to grow. Cells were also plated in a non-restrictive media that indicated cell viability. Plates were incubated at 37^{O}C in a humidified 5% CO_2 atmosphere for 10-12 days. Following incubation all plates were scored for total number of colonies/plate. The frequency of mutation by dose was determined by comparing the number of colonies in the mutagenicity plates to the number of colonies in the corresponding viability plates. For the study to be acceptable the following criteria must be met: mutation frequency of positive controls with or without activation should be twice that of the solvent control; the negative control spontaneous mutation frequency should be in the range of 10 to 100/10^6 cells; negative control plating efficiency should be at or above 50%. The test material should be tested to the level of approximately 10% total growth, or the limits of solubility or to a high dose of 10 mg/ml. The following criteria were used as guidelines in judging the significance of test material activity: Positive – the mutation frequency of one or more test material concentrations, with $\geq$10% total growth, is $\geq$ 2 times the average mutation frequency of the negative controls. Negative - none of the dose levels exhibit a 2x increase in mutation frequency over background (solvent control).
Results	The test substance was not mutagenic in this assay with or without metabolic activation.
Remarks	In the mutagenicity assay the test material was diluted with 5% Pluronic F-68 (w/w in distilled water) and tested over a range of concentrations to the limit of solubility. After the two-day expression period, 5 cultures with metabolic activation and 3 and 5 cultures without activation were selected for cloning based on the degree of observed toxicity. Percent total growth ranged from 48% to 89% with activation and 10% to 50% without activation. The positive controls responded appropriately. None of the cultures treated with test material at a range of concentrations up to the limit of solubility, with or without metabolic activation exhibited a mutation frequency that was two times that of the average mutation frequency of the negative controls. Under the conditions of this study the test material was not mutagenic.

Conclusions	The test substance was not mutagenic in this assay with or without metabolic activation.
Data Quality	Reliable without restriction (Klimisch Code)
References	Unpublished confidential business information
Other	Updated: 8/7/01

Robust Summary #: 25-GenTox-3

Test Substance	
CAS #	CAS# 84605-20-9
Chemical Name	Bis alkenyl succinimide derivative
Remarks	Test material purity not provided.
Method	
Method/Guideline followed	OECD Guideline 474
Test Type	Mammalian Erythrocyte Micronucleus Test
GLP (Y/N)	Y
Year (Study Performed)	1996
Species	Mouse
Strain	Swiss Albino Crl:CD-1 (ICR)BR, 57 days of age at initiation of dosing
Route of administration	Intraperitoneal
Duration of test	Single dose followed by 72-hour evaluation period.
Doses/concentration levels	0, 1250, 2500, 5000 mg/kg
Dose volume	10 ml/kg
Sex	Males and females
Frequency of treatment	Once
Control and treatment groups	Peanut oil vehicle control: 15/sex; Cyclophosphamide positive control: 60 mg/kg (in sterile water), 5/sex; 1250, 2500, 5000 mg/kg: 15/sex.
Statistical methods	Data summarized by sex and dose group/time point. Analysis performed using an analysis of variance, Dunnett's test, and Cochran-Armitage test for linear trend
Dose Rangefinding Studies	Two dose range finding studies were conducted at the following dose levels: 1) 41.2, 412, 4120 mg/kg; 2) 1625, 2750, 3875, 5000 mg/kg; Animals observed for signs of toxicity and mortality.
Remarks field for test conditions	All animals were observed immediately after dosing and periodically throughout the study for toxic signs and/or mortality. Five/sex from each treatment group and vehicle control group were sacrificed for bone marrow sampling 24, 48 and 72 hours post treatment. Positive controls were sampled at 24 hours only. Necropsies were not performed. Bone marrow smears were scored for micronuclei and the polychromatic erythrocyte (PCE) to normochromatic erythrocyte (NCE) cell ratio. 1000 PCEs were scored per animal (Guideline calls for 2000/animal to be evaluated. This difference from the current guideline was not considered sufficient to affect the reliability of the study.) The frequency of micronucleated cells was expressed as percent micronucleated cells based on the total PCEs present in the scored optic field. The frequency of PCEs vs NCEs was determined by scoring the number of each observed in the optic field while scoring at least the first 1000 erythrocytes. If the test article induced neither a statistically significant dose response nor a statistically significant increase at any dose level above concurrent vehicle, at any sampling point, it was considered negative.
Results	
Remarks	Based on the data observed in the rangefinding studies the maximum tolerated dose was estimated to be 5000 mg/kg. Dose levels selected for the main study were 1250, 2500 and 5000 mg/kg

	All vehicle and positive control and 1250 mg/kg/day animals were normal after dosing and remained healthy until the appropriate harvest times. The 5000 mg/kg males and females were slightly hypoactive at 19 and 42 hours post dosing. This finding was also observed at 66 hours in the 5000 mg/kg males only. At 42 and 66 hours post dosing the males at this dose level also exhibited rough hair coats. Females were normal. The 2500 mg/kg males were slightly hypoactive at 42 and 66 hours post dosing. At 66 hours post dosing the males at this dose level also exhibited rough hair coats. Females were normal. Some evidence of bone marrow toxicity was observed as the test material did induce statistically significant decreases in the PCE: NCE ratio in the 2500 mg/kg males and 5000 mg/kg females at the 72 hour evaluation interval and in the 5000 mg/kg males at 48 and 72 hours. A statistically significant increase in micronucleated PCEs was observed in the 5000 mg/kg males at 24 hours. This increase was attributed to the low number of micronucleated PCEs in the concurrent control group. In addition there was no dose response and the value (0.1%) was within the historical control range for the laboratory. No other statistically significant increases in micronucleated polychromatic erythrocytes over the levels observed in the vehicle controls occurred at any of the other harvest times. The positive control induced statistically significant increases in micronucleated PCEs in both sexes compared to the vehicle controls.
Conclusions	The test material was considered negative under the conditions of this study.
Data Quality	Reliable without restriction (Klimisch Code)
References	Unpublished confidential business information
Other	Updated: 8/8/01

Robust Summary #: 25-GenTox-4

Test Substance	
CAS #	CAS# 84605-20-9
Chemical Name	Bis alkenyl succinimide derivative
Remarks	Test material purity not provided.
Method	
Method/Guideline followed	OECD Guideline 471
Test Type	Bacterial Reverse Mutation Assay
GLP (Y/N)	Y
Year (Study Performed)	1996
Test System	*Salmonella typhimurium* and *Escherichia Coli*
Strains Tested	*Salmonella typhimurium* tester strains TA98, TA100, TA1535, TA1537; *Escherichia Coli* tester strain WP2*uvr*A
Exposure Method	Plate incorporation
Test Substance Doses/concentration levels	Initial assay: *Salmonella* + (S9): 100, 250, 500, 1,000, 5,000 and 10,000 ug/plate *Salmonella* - (S9): 100, 250, 500, 1,000, 5,000 and 10,000 ug/plate WP2*uvr*A + (S9): 100, 250, 500, 1,000, 5,000 and 10,000 ug/plate WP2*uvr*A - (S9): 100, 250, 500, 1,000, 5,000 and 10,000 ug/plate Confirmatory assay: *Salmonella* + (S9): 100, 250, 500, 1,000, 5,000 and 10,000 ug/plate *Salmonella* - (S9): 100, 250, 500, 1,000, 5,000 and 10,000 ug/plate WP2*uvr*A + (S9): 100, 250, 500, 1,000, 5,000 and 10,000 ug/plate WP2*uvr*A - (S9): 100, 250, 500, 1,000, 5,000 and 10,000 ug/plate
Metabolic Activation	With and without (500 ul of 10% S9 fraction mix of livers of Aroclor 1254 pretreated Sprague Dawley rats)
Vehicle	Pluronic F127 (25% w/w in ethanol)
Vehicle Control	Pluronic F127 (25% w/w in ethanol)
Tester strain, activation status, Positive Controls and concentration level	TA98 +S9 benzo(a)pyrene 2.5 ug/plate TA98 -S9 2-nitroflourene 1.0 ug/plate TA100 +S9 2-aminoanthracene 2.5 ug/plate TA100 -S9 sodium azide 2.0 ug/plate TA1535 +S9 2-aminoanthracene 2.5 ug/plate TA1535 -S9 sodium azide 2.0 ug/plate TA1537 +S9 2-aminoanthracene 2.5 ug/plate TA1537 -S9 ICR-191 2.0 ug/plate WP2*uvr*A +S9 2-aminoanthracene 25.0 ug/plate WP2*uvr*A –S9 4-nitroquinoline-N-oxide 1.0 ug/plate
Statistical Analysis	Mean revertant colony count and standard deviation were determined for each dose point.
Dose Rangefinding Study	Conducted using tester strains TA100 and WP2*uvr*A and ten doses of test material ranging from 10.0 to 10,000 ug/plate, one plate/dose with (10% S9 homogenate/ml of S9 mix) and without metabolic activation. Cytotoxicity was evaluated.
S9 Optimization Study	Conducted using tester strains TA98 and TA100, and a non-cytotoxic dose level of test article (10,000 ug/plate) and four concentrations of S9 mix (5, 10, 20 and 80% S9 homogenate/ml of S9 mix). Cytotoxicity was evaluated.

Remarks field for test conditions	In the main study there were two treatment sets for each tester strain, with (+S9) and without (-S9) metabolic activation. Each of the tester strains was dosed with six concentrations of test substance, vehicle controls, and a positive control. Three plates/dose group/strain/treatment set were evaluated. The results of the initial assay were confirmed in a second independent experiment. 50 ul of test material, positive control or vehicle control were added to each plate along with 100 ul of tester strain, S9 mix (if needed) and 2.0 (with S9) or 2.5 ml (without S9) of top agar. This was overlaid onto the surface of 25 ml minimal bottom agar in a petri dish. Plates were incubated for 48 hours at 37^{o}C. Plates that were not evaluated immediately were held at 5^{o}C until evaluated. The condition of the bacterial background lawn was evaluated for cytotoxicity and test article precipitate. The number of revertant colonies/plate for the vehicle controls and all plates containing test article were counted manually. The number of revertant colonies/plate for the positive controls were counted by automated colony counter.
Results	The test substance was not genotoxic in this assay with or without metabolic activation.
Remarks	In the dose rangefinding study no cytotoxicity was observed with tester strain TA100 or WP2*uvr*A at dose levels up to 10,000 ug/plate with or without metabolic activation. Test article precipitate was observed on plates at 3,330 ug/plate and above with tester strain TA100 and WP2uvrA with and without metabolic activation. Based on these results the dose levels outlined above (page 1, Test Substance Doses, Initial Assay) were selected. The S9 optimization study was performed using TA98 and TA100 with a non-cytotoxic dose of test article, (10,000 ug/plate) and four concentrations of S9 mix (5, 10, 20 and 80% S9 homogenate/ml of S9 mix). In the absence of any effect a 10% S9 mix was used in the mutagenicity study. In the initial assay all data were acceptable and no positive increases in the number of revertants/plate were observed. The confirmatory assays were conducted using the same dose levels. In these confirmatory mutagenicity assays all data were acceptable and no positive increases in the number of revertants/plate were observed with any of the tester strains with or without metabolic activation. However, the vehicle control values for three tester strains (TA98, 1535 and 1537) were higher then routinely expected with metabolic activation. Based on these results these three tester strains were retested for confirmation again. In these confirmatory mutagenicity assays all data were acceptable and no positive increases in the number of revertants/plate were observed. Vehicle control values were within routinely expected values. Based on these results the test material was considered not mutagenic. No cytotoxicity was observed up to 10,000 ug/plate with the *Salmonella* tester strains with and without activation and with WP2*uvr*A with and without activation. Test material participate was observed on plates at $\geq$500 ug/plate. The positive control for each respective test strain exhibited at least a 3-fold increase (with or without S9) over the mean value of the vehicle

	control for a given strain, confirming the expected positive control response.
Conclusions	Under the conditions of this study, the test material was not mutagenic.
Data Quality	Reliable without restriction (Klimisch Code)
References	Unpublished confidential business information
Other	Updated: 8/6/01